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14. Abstract: Optimal early detection and prevention strategies for breast cancer are predicated on our ability to identify individuals at significantly increased risk for this disease. The purpose of this Center is to bring molecular risk prediction for breast cancer into the clinical area. This will require progress on three fronts of scientific endeavor: (i) Establishment of a tissue repository of benign breast disease; (ii) Assessment of potential biomarkers of risk in this tissue set and (iii) Discovery of new, potentially relevant biomarkers of risk. We have made significant progress on these aims. Our current cohort comprises 9,376 women, 758 (8%) of whom have been diagnosed with breast cancer since the time of their benign biopsy. We established our tissue repository of benign breast tissue and have collected the subsequent breast cancers for 646/758 (85%) of the cases. We assessed the significance of benign histology in predicting risk of future breast cancer, examining in detail the role of proliferative disease, atypia, papillomas, radial scars and involution. We explored the link between centrosome amplification and COX-2 and breast cancer outcomes. We will be focusing this upcoming year on additional molecular markers and genetic profiling.					
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**Benign Breast Disease: Toward Molecular Prediction of Breast Cancer Risk**

**INTRODUCTION**

Our fourth year Center of Excellence report details a total of 40 months of work involving human subjects. Delays during initial approval processes led to some delay in the start-up funding for the human subjects portion of the grant.

Three main aims of scientific activity exist within our Center of Excellence: 1) the establishment of a large tissue repository from a retrospective cohort of women with benign breast disease (BBD) (1967-1991); 2) the application of potential biomarkers of risk to this archival tissue set; and 3) the discovery of new, potentially relevant biomarkers of risk in fresh and frozen specimens of BBD. The Center includes a multi-institutional team of basic scientists, pathologists, epidemiologists, clinicians, statisticians, and advocates (Mayo Clinic; University of California San Francisco (USCF); Wayne State).

**I. Task 1: Establish Retrospective Cohort of BBD and Nested Case-Control Study**

**A. Complete cohort follow-up**

We provide here an update of our cohort. Numbers have been refined as we have collected additional data from questionnaires, charts and pathology readings.

Our total cohort now includes 9,376 women—we have follow-up data and benign tissue for all. 758 (8%) of them have been diagnosed with breast cancer since the time of their benign biopsy. 7213 (77%) of these women are alive. The median time from benign breast biopsy to the diagnosis of breast cancer is 11 years. We received questionnaire data on 5,692 (61%); 646 (11%) of these were completed by next-of-kin.

**B. Validate reported breast cancers**

To validate reported breast cancers, charts were reviewed on women diagnosed at Mayo Clinic. For women outside of Mayo Clinic, a contact was initiated to obtain permission to access medical records associated with their breast cancer diagnosis and their breast cancer tissue.

For the 758 women diagnosed with cancer, 427 (56%) were diagnosed at the Mayo Clinic, while 331 (44%) were diagnosed outside of the Mayo Clinic. We have been successful in obtaining blocks on 407/427 (95%) of the women diagnosed at Mayo Clinic and 194/331 (59%) diagnosed outside of the Mayo Clinic. We have slides on an additional 5 (1%) of the women diagnosed at Mayo Clinic and 40 (12%) diagnosed outside of Mayo Clinic. Thus, altogether we have either slides or blocks from the breast cancers for 646 (85%) of the 758 women. No tissue has been

obtained for 112/758 (15%) of women diagnosed with cancer. Of these women, we did not request permission on 40/112 (35%) as they or their next-of-kin did not complete the questionnaire. Additionally, 38/112 (34%) of these women or their next-of-kin did not grant

**Abbreviations**

BBD = Benign breast disease  
NP = Non-proliferative  
PDWA = Proliferative disease without atypia  
AH = Atypical hyperplasia  
RR = Relative risk  
TDLUs = Terminal duct lobular units  
IHC=Immunohistochemistry

permission for their tissue to be released to us. No tissue is available for the remaining 34 women.

This past year, our study pathologist has characterized type of cancer for the majority of our cancer tissue. Thus far, he has reviewed 468 (72%) of the 646 obtained cancer tissues. The histologic subtypes for the 468 cancers are shown below.

<b>Cancer Type</b>	<b>Number (%)</b>
<b>Invasive</b>	<b>369</b>
Infiltrating Ductal	239 (62%)
Infiltrating Lobular	45 (12%)
Mixed Ductal/Lobular	50 (13%)
Tubular	9 (2%)
Mucinous	10 (3%)
Medullary	3 (1%)
Papillary	8 (2%)
Metaplastic	3 (1%)
Tubulolobular	2 (1%)
<b>In-Situ</b>	<b>99</b>
DCIS Only	91 (92%)
LCIS Only	1 (1%)
DCIS & LCIS	7 (7%)

\*Invasive characteristics  $\geq 100\%$  as may cancer may be represented by more than one characteristic

C. Match appropriate controls to known breast cancer cases

We described this process in our 2004 report. This task has been completed.

D. Construct test set for preliminary evaluation of markers

We described the construction of our test set in our 2004 report. This subset consists of 124 cases and their two closest controls selected from the entire study period.

E. Construct validation set from remaining breast cancer cases, each matched with two controls

The remaining cases and controls will serve as the validation set.

II. **Task 2: Biomarkers in Archived Tissues from Cases and Controls**

A. Retrieve tissue slides/blocks of BBD specimens for all cases and controls

We were able to retrieve archived benign tissue blocks from the Mayo Clinic Tissue Registry for 658 (87%) of the cases. We obtained slides for an additional 110 (13%). We have also collected tissue blocks on two matched controls for each case.

B. Characterize benign histopathology

1. General findings

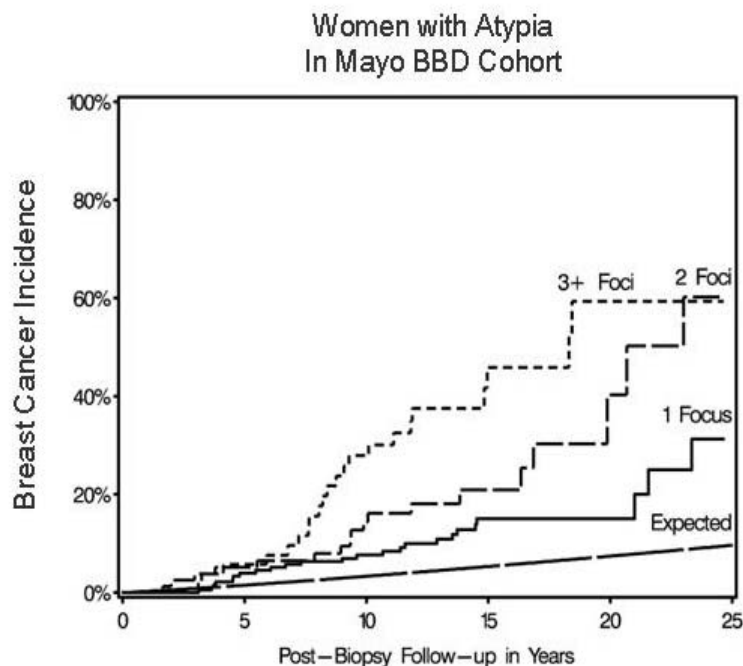
Last year we reported the benign histology for our entire cohort. This objective has been completed.

We published our first manuscript July 2005 in the *New England Journal of Medicine*. In this manuscript, reporting on a cohort of 9,087 women, we examined how family history, pathology and age affected women's risk of subsequent breast cancer. We found the following:

- The broad histologic classifications included non-proliferative disease (NP), 6061 (66.7%); proliferative disease without atypia (PDWA) 2690 (29.6%); and atypical hyperplasia (AH) 336 (3.7%).
- The relative risks for a subsequent breast cancer were: NP = 1.27; PDWA = 1.88 and AH = 4.24
- The relative risks by family history categories were: None = 1.18; weak = 1.43 and strong = 1.93
- Risks of ipsilateral vs contralateral breast cancer varied by histology and years after benign biopsy (see manuscript for details)

## 2. Atypia

We have studied our cohort of women with atypia in depth and these findings have been submitted for publication. We found that the risk of breast cancer was elevated for women with atypia and even greater for women with atypia who were under age 45 (RR=7.36). We examined risk by number of foci of atypia and found: 1 focus, RR=2.33; 2 foci, RR=5.41; and for three or more foci, RR=7.96 (see cumulative incidence figure below). Moreover, in the highest risk subgroup of women with three or more foci of atypia and histologic calcifications, the cumulative incidence exceeded 50% after 25 years. This level of risk approaches that



Observed cumulative breast cancer incidence among women with atypical hyperplasia, stratified by number of foci of atypia. Expected events calculated by applying age- and calendar period-stratified person years of observation among all women with atypia to corresponding Iowa SEER breast cancer incidence rates. Observed and expected events cumulated using Kaplan-Meier product limit methodology. (From Degnim AC, Visscher D, . . . Hartmann LC. Stratification of breast cancer risk in women with atypia: A Mayo cohort study, submitted 2006).

reported for carriers of BRCA1/2 mutations. These data were presented at the Society of Surgical Oncology in 2005 and a manuscript has been submitted for review.

## 3. Papillomas

The risk of breast cancer development in patients with papillomas, particularly those with multiple or atypical lesions, has been incompletely defined. We examined the association between breast papillomas and subsequent risk of breast cancer. We found that a single

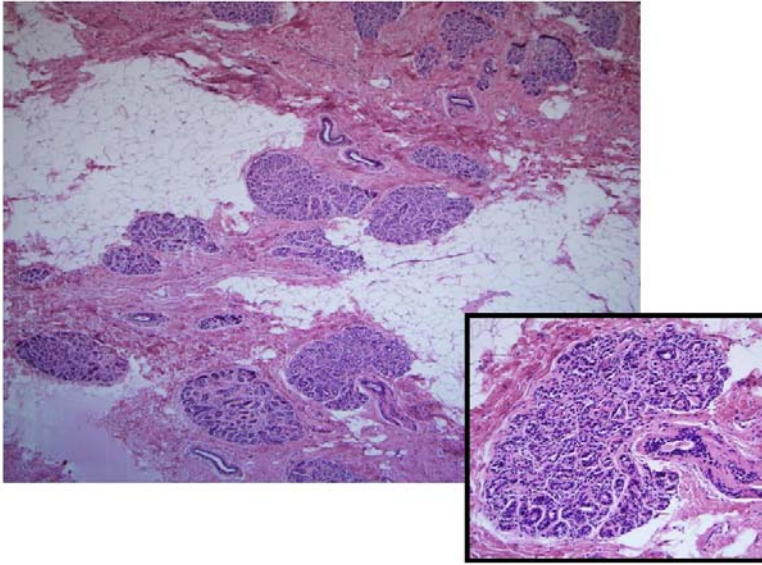
papilloma imparts a cancer risk similar to conventional proliferative fibrocystic disease. The presence of single papilloma with atypia does not modify the risk of atypical ductal hyperplasia/atypical lobular hyperplasia overall. The presence of multiple papillomas, however, increases the risk of breast cancer over that of proliferative fibrocystic disease (RR 3.01, 95% CI 1.10-6.55), even more so in women with multiple papillomas with atypia (RR 7.01, 95% CI 1.919-17.97). Thus, multiple papillomas constitute a proliferative breast disease subset having unique clinical and biologic behavior. These data were presented at the United States and Canadian Academy of Pathology in San Antonio, TX, February 28, 2005. The following manuscript has been published.

Lewis JT, Hartmann LC, Vierkant RA, Maloney SD, Pankratz VS, Allers TM, Frost MH, Visscher DW. An Analysis of Breast Cancer Risk in Women with Single, Multiple, and Atypical Intraductal Papilloma. American Journal of Surgical Pathology. 2006: 30;665-672.

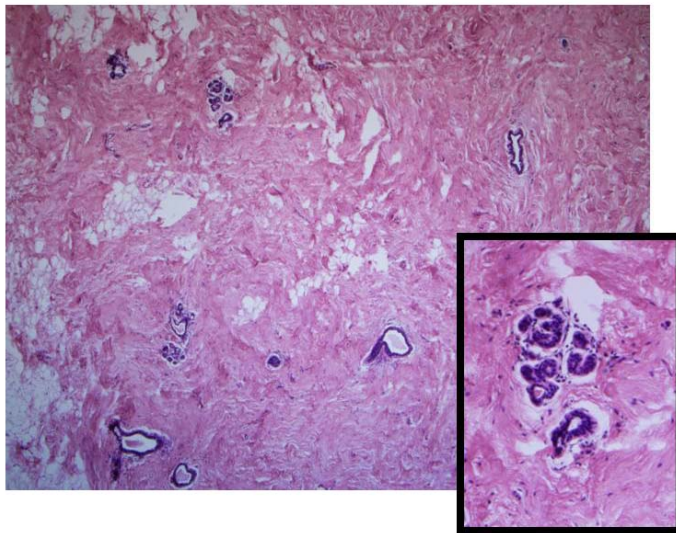
#### 4. Involution

There are very few pathologic features that are associated with a reduced risk of breast cancer. In our BBD resource, we studied if regression or involution of a woman's breast lobules (or terminal duct lobular units, TDLUs) was associated with later risk of breast cancer. The breast is organized into approximately 15-20 major lobes, each made up of lobules that contain the milk-forming acini. As a woman ages, these lobules regress or involute with a reduction in the number and size of acini per lobule (see figure).

(a)



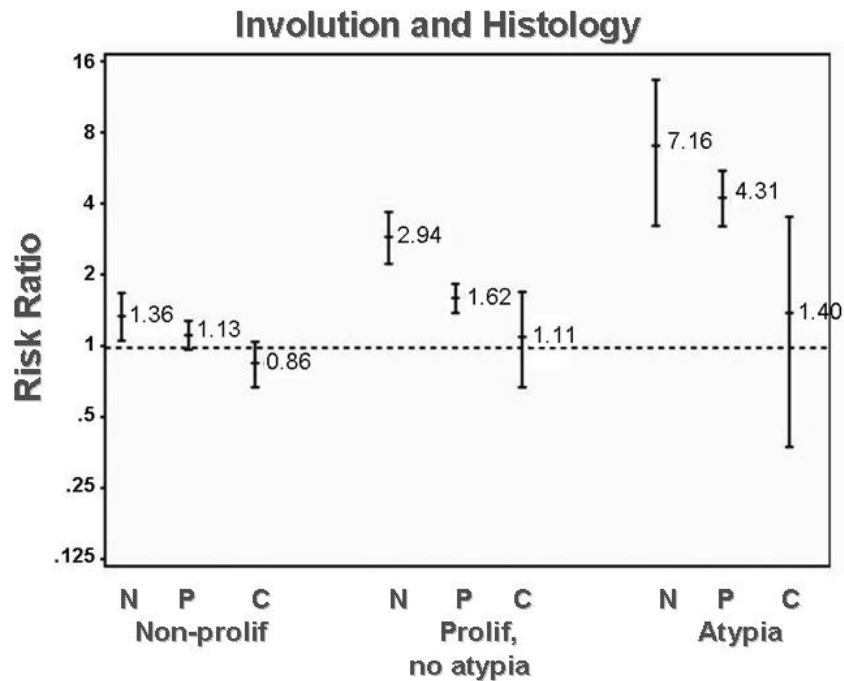
(b)



Histologic features of age-related involution. (a) An example of pre-involutorial breast tissue with multiple intact lobules, each comprised of multiple acini and specialized stroma (inset). (b) An example of complete lobular involution showing mostly residual ducts with residual lobules, largely depleted of acini (inset).

Our study pathologist assessed the extent of involution in the background breast tissue of the women in our BBD cohort. Notably, those women who had complete involution of their TDLUs had a significantly lower risk of breast cancer compared to those with partial or no involution. We found that the presence of complete involution reduced risk even in women who were at high risk because they had atypia or a strong family history of breast cancer. This is a novel finding because the subject of involution has not been studied in the human in the past several decades. Importantly, this provides an additional feature to assess on a breast biopsy that allows us to finetune risk prediction for women. Secondly, and even more importantly, if the scientific community can determine what controls the process of breast involution, we may be able to induce it medically and thus introduce a new “chemoprevention” strategy to offer women. These data were presented at the United States and Canadian Academy of Pathology Annual Meeting in Atlanta, GA, February, 2006. A manuscript has been submitted for review.





In this diagram, we show the effect of progressive degrees of involution, from none (N) to partial (P) to complete (C) on the risk of breast cancer associated with various histologic categories of BBD. If a woman has proliferative disease without atypia and complete involution of background TDLUs, her risk of breast cancer is not increased (vs. a RR of 2.94 if no involution). Women with atypia and complete involution have a RR of 1.4, vs. 7.2 with no involution. Some confidence intervals are wide, underscoring the need to study additional patients.

#### C. Prepare tissue slides for biomarker analyses

Tissue slides have been prepared for the test set and two other subgroups of interest: women whose breast cancer occurred within 5 years of their diagnosis (n = 174) and women whose histopathology revealed atypia (n = 336).

#### D. Perform IHC of molecular markers

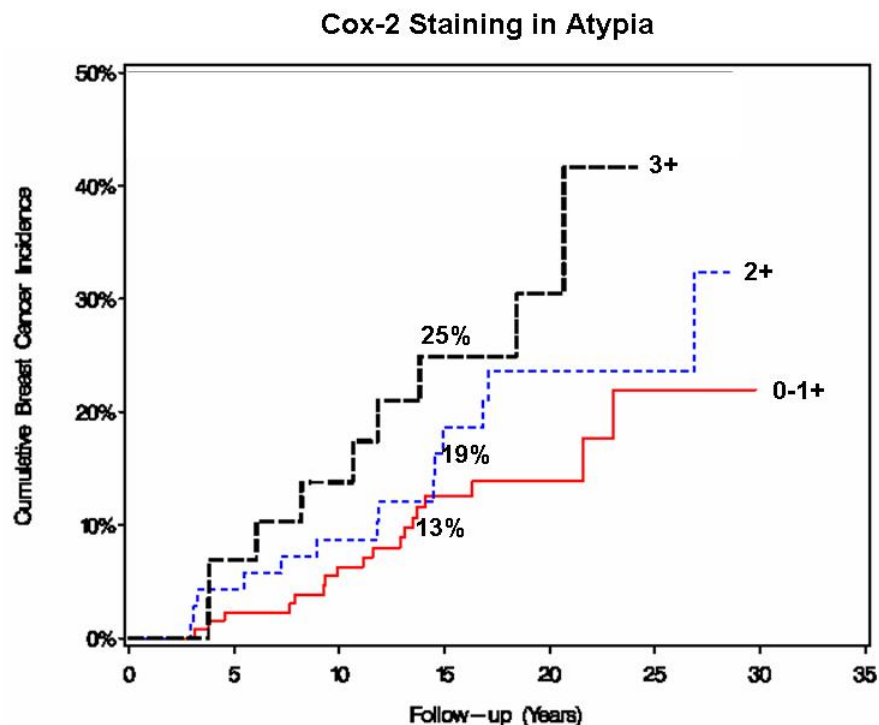
Our focus continues to be on the earliest possible changes that we might detect in these "pre-malignant" lesions. During the June 2005 DOD Era of Hope meeting, there was much discussion on the identification of pre-malignant lesions and possible biomarkers to study. There is certainly no consensus on this point. Our decision was to begin with COX-2, ER alpha, MIB-1, gamma tubulin and cyclin-D, and the test set and atypia subgroup have been stained for these markers.

##### 1. COX-2

COX-2 is a very important mediator of biologic processes during inflammation and cancer. Through work of our UCSF study team led by Dr. Thea Tlsty and other labs, we know that COX-2 expression is up-regulated in invasive breast cancer and also in ductal carcinoma in situ. We sought to determine if increased expression occurred a step earlier — namely in women with atypia — and if the presence of high levels of COX-2 would predict which women with atypia would go on to develop breast cancer. In fact, we found that moderate to strong COX-2 expression is associated with a significantly greater likelihood of a subsequent breast cancer in women with atypia. (See figure below). For women whose atypia lesion exhibited negligible (0-

1+) staining, their likelihood of developing breast cancer was 13% at 15 years from biopsy, vs. 25% for those with 3+ COX-2 staining.

Besides its potential for risk prediction, COX-2 represents a molecular target for chemoprevention strategies. COX-2 inhibitors are available pharmaceutically and in fact, epidemiologic studies have shown that women who have taken COX-2 inhibitors for arthritis have a lower chance of developing breast cancer. Our data regarding COX-2 expression in women with atypia were presented at the 2006 Annual Meeting of the American Association of Clinical Research held in Washington, D.C., in April. This work was featured recently in *JAMA News* and this is included as an Appendix.



## 2. Other IHC analyses

We are proceeding with several other candidate marker analyses. Specifically, our study pathologist is currently quantifying ER alpha levels in the atypia set. A computerized program is being developed to quantify the scores with the proliferation marker MIB-1.

Regarding the originally proposed studies with FISH, we are pursuing a conservative approach overall. Clearly these small benign lesions are a valuable resource and one that we want to preserve for the most promising markers.

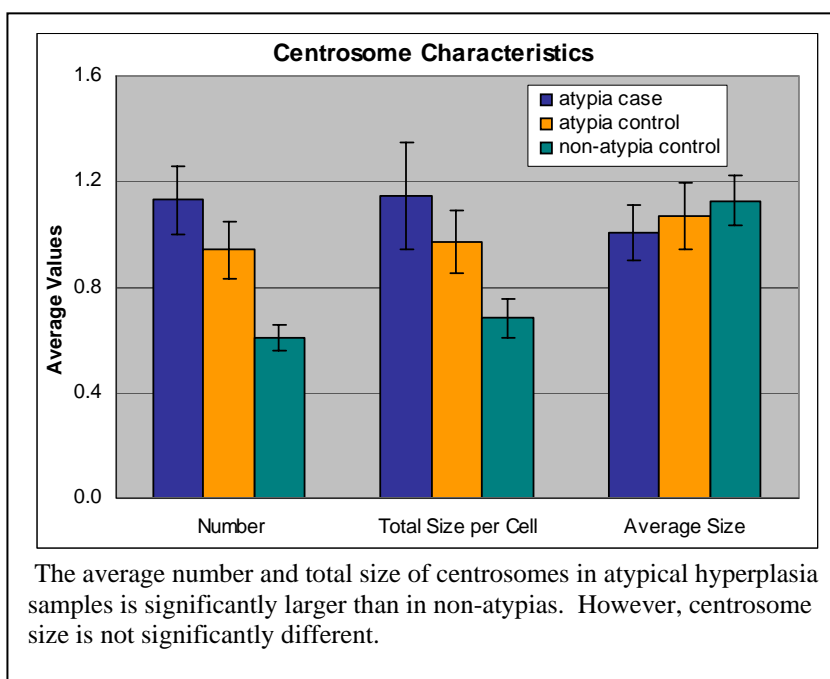
## E. Perform centromere studies.

Most invasive breast cancers, like many other solid tumors, have amplified centrosomes. The extent of centrosome amplification correlates with the levels of chromosomal instability in invasive ductal carcinoma of the breast. Centrosome amplification is also present in ductal

carcinoma in situ, but has not been investigated in benign breast lesions. In this study we are investigating the status of centrosomes in benign breast lesions of various histologies to determine if amplified centrosomes can be detected in the absence of malignancy and invasion, and if any histologic types of benign breast lesions have significant levels of centrosome amplification.

In our previous progress report we showed through image analysis of immunofluorescent labeling of gamma tubulin in paraffin sections, that centrosome amplification was seen infrequently in non-proliferative lesions and in proliferative lesions without atypia. However, about 88% of atypical hyperplasia lesions had detectable centrosome amplification, with about 30% exhibiting moderate to considerable levels of centrosome amplification. Thus, centrosome amplification is seen more frequently in benign lesions having the highest relative risk of developing breast cancer.

A nested case control study investigating centrosome amplification in patients with atypical hyperplasia is now underway. In this study we are measuring centrosome amplification in 20 patients with atypical hyperplasia who developed invasive cancer within 6 years (cases) and 40 patients with atypia who did not develop invasive cancer within that time period (controls). For each case there are two controls matched on age at diagnosis of atypia and the year of the diagnostic biopsy. We are measuring



centrosome number per cell, average centrosome size, and average total size per cell. Thus far we have analyzed 14 cases and 24 controls. Our results to date are shown in the bar graph, along with previous data from non-proliferative lesions. We see a trend for increased centrosome number and total size per cell in the cases compared to the controls. Interestingly, there is no difference in the average size of centrosomes between the 3 groups of patients. This indicates that an increase in centrosome number, but not size, may be an early event in the process of breast carcinogenesis. Full statistical analysis will be done on the completed case control study.

### III. Task 3: Discovery - *In Vitro* Culturing and Gene Profiling Studies

A. Identify appropriate frozen proliferative BBD specimens at Mayo and Wayne State for profiling.

The purpose of these studies is to identify new, potentially relevant biomarkers in benign breast disease, markers that might correlate with subsequent breast cancer risk. When our grant was submitted, the technology was not available to do profiling studies in paraffin-embedded tissue (such as our BBD resource) and hence, we described doing profiling in frozen samples of BBD. A serious limitation of that approach, however, is that we do not have outcome information for our frozen repository samples, since these were accrued recently, and insufficient time has elapsed for the development of breast cancer. Fortunately, genomic profiling technology has proceeded significantly and there now are platforms available for us where microdissected, paraffin-embedded samples can be run. We are working currently to identify the quantity and quality of DNA and RNA that can be obtained from the paraffin-embedded samples.

**B. Obtain fresh BBD tissue from appropriate patients at Mayo and Wayne State for culturing in vitro at UCSF.**

Forty-four samples were sent from Mayo to UCSF. Five of these samples were lost to contamination.

Multiple efforts to implement a prospective collection of fresh tissue in African-American women at Wayne State proved to be logistically impossible to launch. Thus, we have moved to develop a retrospective study in an African-American cohort at Wayne State, modeled after the Mayo (Caucasian) cohort. Through a collaboration with Dr. Hind Nassar, a junior pathologist at Wayne State, an IRB-approved protocol has been developed, to access paraffin-embedded samples of benign breast disease (BBD) from African-American women at Wayne State from 1992-2001. This will allow us to begin to look at the problem of BBD in African-American women. Moreover, because the population there is covered through the Detroit SEER database, we will have information about cancer outcomes.

**C. Culture BBD specimens and document their growth characteristics**

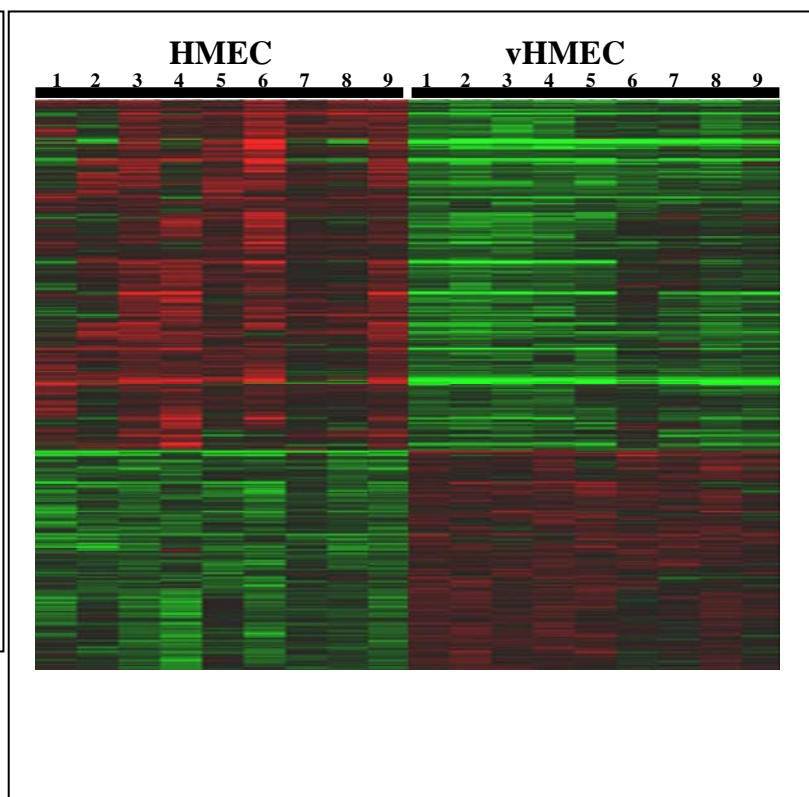
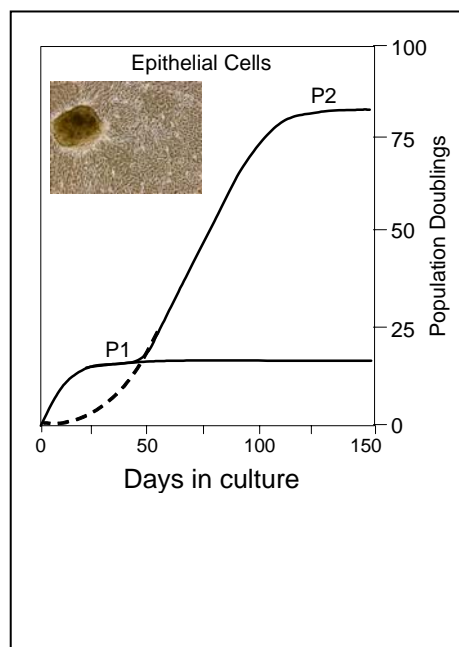
These data were reported last year.

**D. Compare genomic expression levels of DCIS markers in BBD tissues.**

We described in our grant proposal using DCIS samples as a springboard for the identification of potentially relevant biomarkers in BBD. We have identified a cohort of 155 women who had DCIS diagnosed and treated at Mayo in the 1970s and early 1980s. We have successfully created a tissue microarray from these samples for marker testing. Markers that prove to be promising in DCIS samples can then be considered for testing in the BBD samples.

**E. Profile BBD specimens.**

We have isolated and propagated epithelial cells from disease-free breast tissue and tissue containing benign breast disease to determine the growth kinetics of BBD epithelial cells. To date all BBD tissue generated two epithelial populations with distinct growth characteristics, similar to epithelial cells generated from disease-free breast tissue. Briefly, the first population of human mammary epithelial cells (HMEC) grows in culture for approximately 10-15 population doublings before reaching an irreversible p16-dependent growth arrest termed P1. The second population, variant HMEC (vHMEC), grow in culture for an additional 40-50 population doublings due to the loss of p16/Rb signaling before reaching a telomere-dependent growth arrest. We hypothesize the identification of molecular alterations that accompany the extended proliferative capacity of the vHMEC population prior to telomere attrition and genomic instability may provide potential relevant biomarkers of risk. To this end we analyzed the global transcript levels of nine isogenic HMEC and vHMEC populations. Unsupervised hierarchical clustering analysis identified approximately 1240 genes that significantly differentiated the two populations on the

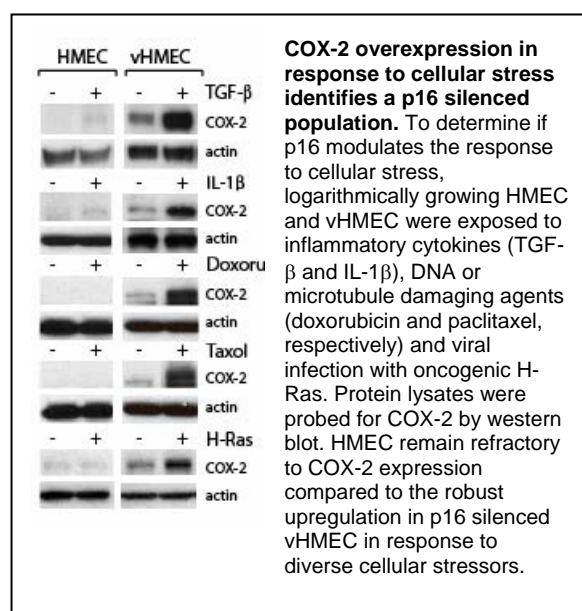


basis of expression patterns. We chose a subset of 512 genes that robustly stratified the two groups (figure above).

Many of the differentially expressed genes in the variant population are known E2F downstream targets, such as survivin, forkhead D1, BUB1 and Rad51. However, many have no known association with p16/Rb signaling, suggesting that the vHMEC are a unique population of cells. We found that many differentially expressed genes in vHMEC resembled expression of genes in DCIS and invasive cancer. These data support the utility of this model for discovery of novel biomarkers for risk assessment. Analysis of global transcript levels is underway for the HMEC isolated from cultured BBD samples accrued to date. Since profiling data are more robust with greater numbers of samples, more analysis is planned.

## COX-2

COX-2 was identified as one of the most robustly upregulated genes in vHMEC. The sustained expression of COX-2 in the vHMEC population is an intriguing finding because COX-2 is a stress activated gene that is tightly regulated in normal cells, such that it is only transiently expressed in response to cellular stress. This finding in the vHMEC cells suggests that this subpopulation exhibit a sustained stress response compared to the majority of epithelial cells. We find that HMEC (normal primary cells) are refractory to COX-2 induction in response to exogenous stress induced by inflammatory cytokines, DNA or microtubule damage, and oncogene-induced stress (adjacent figure). This



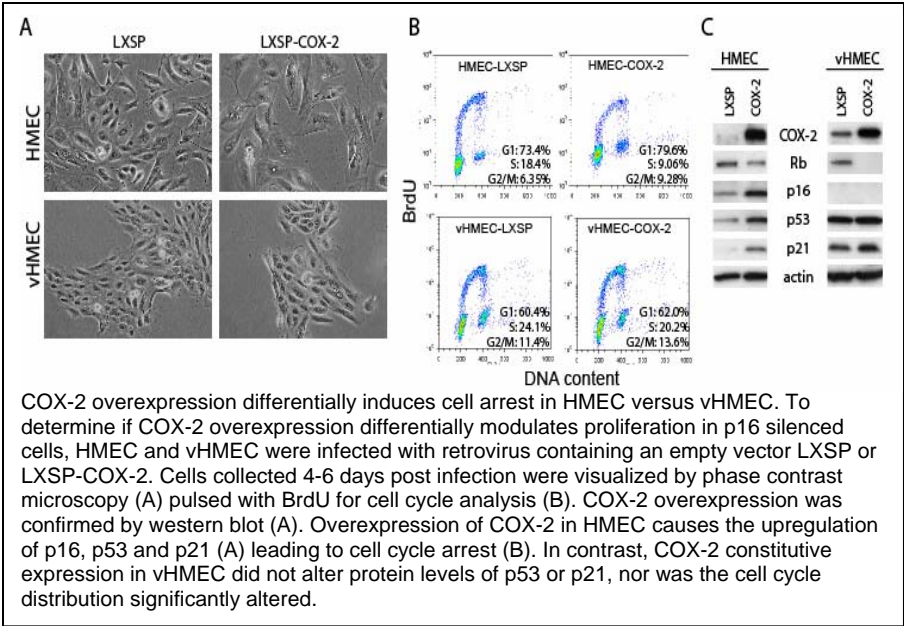


differential induction of COX-2 may reflect a potential for transformation since COX-2 overexpression is

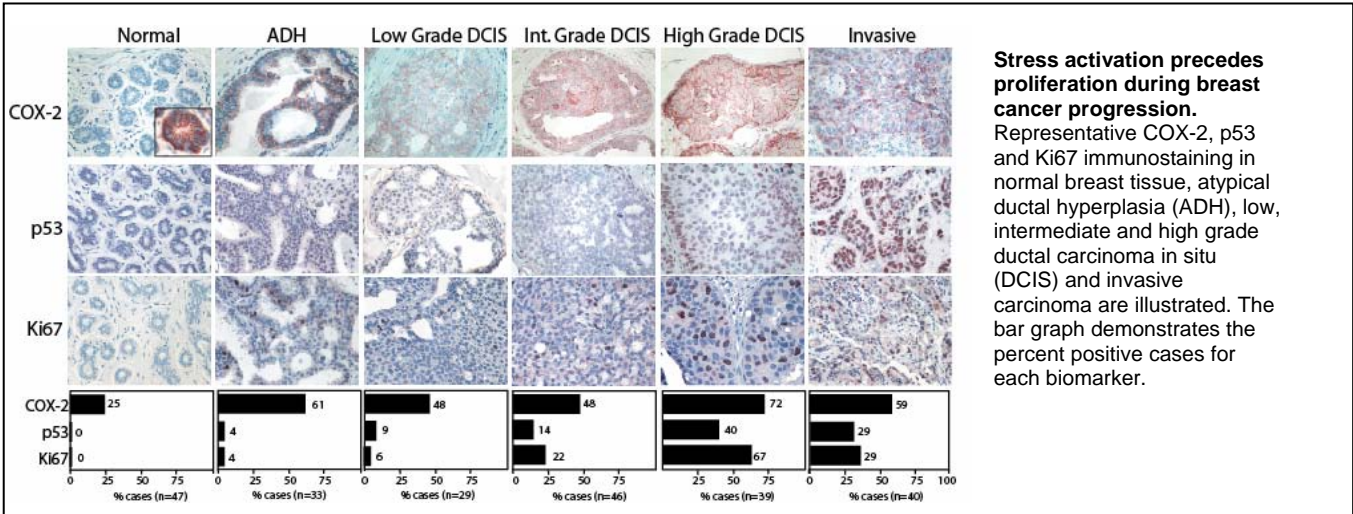
accompanied by phenotypes that are critically relevant to cancer development, such as promoting proliferation, invasion and angiogenesis as well as inhibiting apoptosis and immune surveillance.

This hypothesis is supported by our observations that forced expression of COX-2 in HMEC by retroviral infection produced enlarged flattened cells that were growth arrested (adjacent figure). Cell morphology and proliferation was not altered in vHMEC constitutively expressing COX-2. We find that the molecular changes underlying the differential

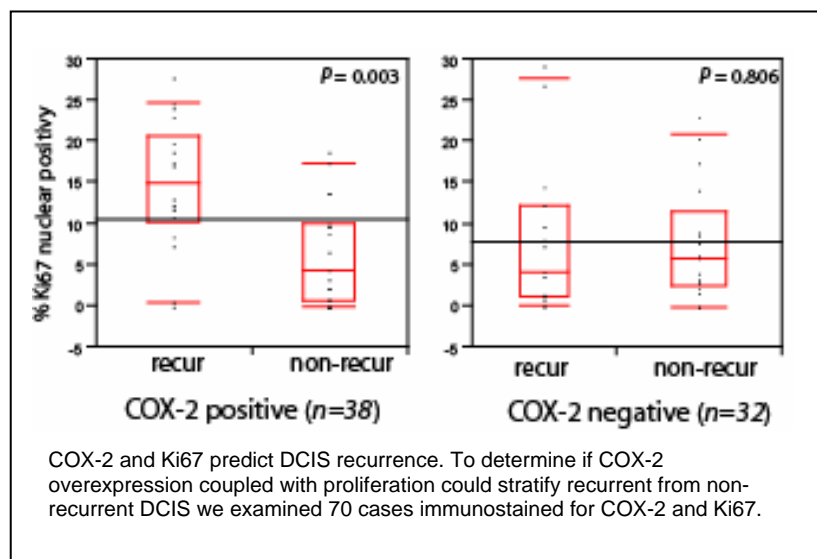
phenotypic response to COX-2 overexpression are dependent on p16/Rb signaling. HMEC overexpressing COX-2 resulted in elevated p16, p53 and p21 and downregulation of Rb (see figure). This is in contrast to p16 silenced vHMEC where overexpression of COX-2 did not alter the level of p53 or p21. Thus, in normal cells, COX-2 induces a cell cycle arrest through the upregulation of p16 and p53 to protect cells from inappropriate oncogenic signaling. In cells that have lost p16/Rb signaling, COX-2 overexpression does not induce a growth arrest. We argue that sustained stress activation in the absence of growth arrest defines an aberrant stress phenotype that may set the stage for carcinogenesis.



To determine if this aberrant stress phenotype is clinically significant, we characterized COX-2 expression through a series of samples that reflect the currently accepted histologic continuum



of breast cancer progression (see below). COX-2 immunopositivity was detected in 61% of ADH lesions, 48% of low and intermediate grade DCIS, 72% of high grade DCIS and 59% of invasive

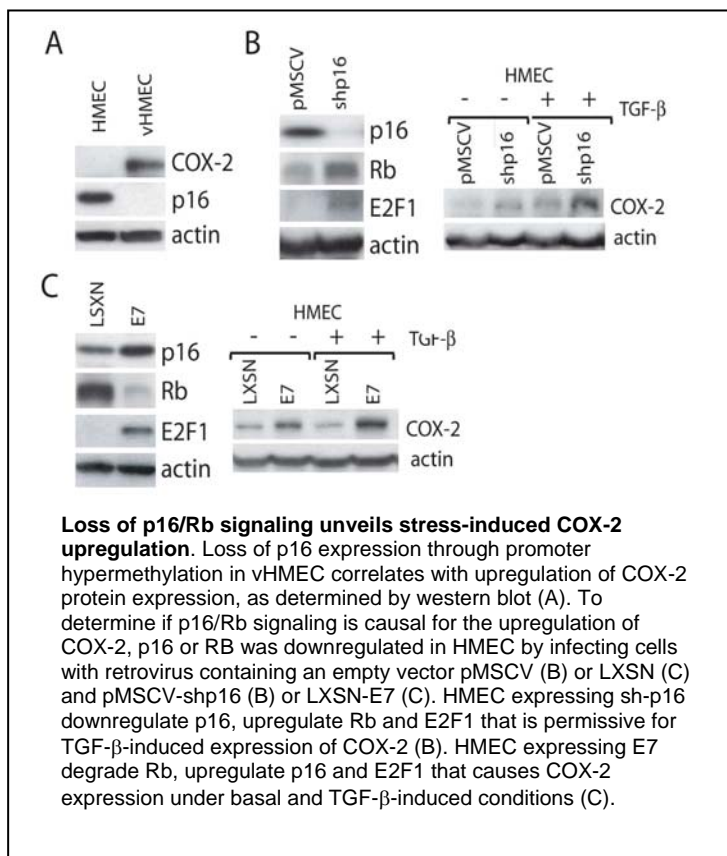


carcinoma . We find that COX-2 overexpression is correlated with DCIS nuclear grade ( $P=0.05$ ). The premalignant lesions display a robust overexpression of COX-2 that was significantly greater than that found in normal breast tissue (25%;  $P < 0.001$ ). Thus, we find the onset of stress activation, as reflected in COX-2 overexpression, occurring prior to active proliferation and the accumulation of p53. One interpretation of these data is that early in premalignancy, COX-2 overexpression may

exert a proliferative arrest and act as an early barrier to breast carcinogenesis. However, in later stages cells have bypassed stress-induced growth arrest while maintaining sustained stress activation. We predict that stressed cells that are actively proliferating may result in clonal selection and outgrowth. To test the clinical relevance of COX-2 overexpression in the setting of breast cancer, we examined a series of 70 recurrent and non-recurrent DCIS cases that were treated with lumpectomy alone. We found that COX-2 and Ki67 immunopositivity could significantly stratify recurrent from non-recurrent DCIS (figure above). Interestingly, 64% of cases that recurred did so as invasive carcinoma.

To further study the relevance of COX-2 in the development of breast cancer, we performed the analysis of COX-2 levels in atypia described under Task 2, and saw a strong link with the subsequent development of breast cancer. Given our observation that COX-2 levels are increased in some normal-appearing breast epithelium, we plan to study COX-2 levels in our non-proliferative and proliferative BBD samples, to test for any correlation with the later development of breast cancer in these earliest lesions.

Further discovery with our HMEC vs vHMEC model system provides us with a biologically relevant model from which potential biomarkers can be identified that can then be tested in the BBD samples with known cancer outcomes. Analysis of the



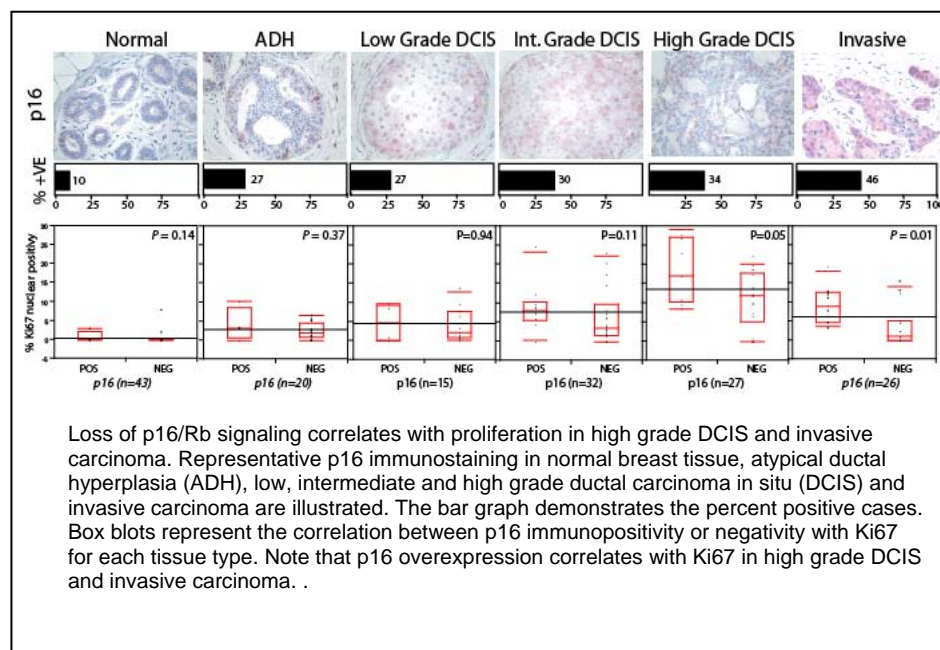
p16/pRb pathway in the human mammary epithelial cells has identified an additional biomarker that may aid in stratification as described below.

## p16

Our in vitro model demonstrated an inverse relationship between p16 and COX-2 expression, as shown in this figure. This finding prompted us to determine if loss of p16/Rb was sufficient to induce COX-2 expression. We find that sequence specific silencing of p16 causes COX-2 upregulation and provides cells with a proliferative advantage. Although genetic downregulation of p16 did not result in robust COX-2 upregulation, cells became responsive to exogenous induction of COX-2 by TGF- $\beta$ , as shown. Since p16 exerts many of its biological effects through Rb, we determined if induction of COX-2 is mediated through Rb. We find that downregulation of Rb by retroviral infection of HMEC with the human papilloma virus E7 (HPV-E7) caused a robust upregulation of COX-2 expression and sensitizes cells to COX-2 induction by exogenous inducers such as TGF- $\beta$ . The absence of Rb also provided a proliferative advantage. Thus, loss of p16 or Rb causes the upregulation of COX-2 and provides cells with a proliferative advantage, thereby mimicking the aberrant stress phenotype described previously in the vHMEC cells. We next sought to determine if p16/Rb signaling is clinically significant.

The majority of normal breast tissue is devoid of p16 immunostaining. Specifically, we observe that only 10% of disease-free tissue contains >30% of the lobules positive for p16. This is in contrast to either pre-malignant or malignant breast lesions. Twenty seven percent of ADH lesions display heterogeneous immunostaining for p16, a significant upregulation ( $P=0.05$ ) compared to normal tissue. This level of immunopositivity and heterogeneity is maintained in low, intermediate and high grade DCIS lesions. The level of p16 positivity in invasive tumors is similar to that observed in DCIS. However, in contrast to DCIS, the pattern of p16 staining in invasive tumors is much more homogeneous.

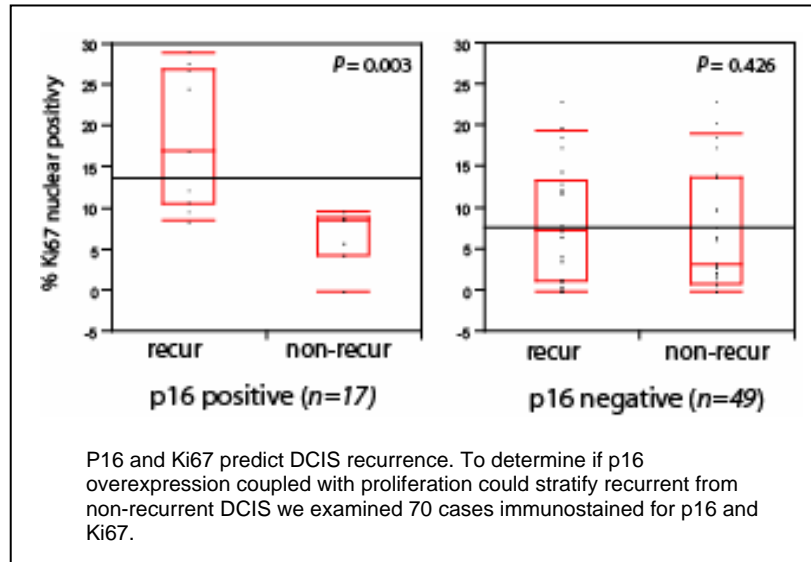
In normal cells overexpression of p16 causes a cell arrest that acts as a protective mechanism in response to diverse cellular stressors or inappropriate mitogenic stimulation. To determine if the upregulation of p16 we observe during pre-malignancy is accompanied by cell arrest, we



determined the relationship between p16 and proliferation in archival tissue samples (see figure). The lesions that stained positive for p16 in normal breast tissue, ADH, low and intermediate grade DCIS showed no relationship with Ki67. These data suggest that p16 may be functionally exerting a cell cycle arrest in

these tissues. In contrast, high grade DCIS lesions and invasive tumors overexpressing p16





were correlated with elevated Ki67 index labeling. The finding that p16 and Ki67 are linked in high grade DCIS and invasive breast tumors strongly suggests that p16-mediated regulation of cell cycle is abrogated. Therefore, p16 overexpression in high grade DCIS and invasive tumors is dysfunctional.

We reasoned that loss of p16/Rb signaling may cause cells to become refractory to stress-induced growth arrest and may reflect a more aggressive phenotype. To determine if p16 overexpression

and Ki67 index labeling could stratify recurrent from non-recurrent DCIS we examined a series of 70 DCIS cases with known outcome. We find that coupling p16 and Ki67 indeed identifies DCIS cases that recur (see figure).

The role of p16 alone or in combination with other biomarkers, is presently being evaluated in the BBD cohort.

#### Task 4: Statistical Analyses

##### A. Establishment of relational database

This task is complete. The database is the foundation for tracking all tissue samples; entering clinical, pathologic, and molecular data; and analyzing results.

##### B. Enter epidemiologic and histopathologic data

This task is complete.

##### C. Enter culturing data (proportion of cells that break through proliferation barriers; slope of curve, etc.)

These data are being entered as collected at UCSF.

##### D. Enter molecular data from culturing experiments (methylation of p16, p53 status, % proliferation versus apoptosis, etc).

These data are being entered as collected at UCSF.

##### E. Enter gene profiling data.

This is ongoing as described in Task 3.

##### F. Calculate hazard function for breast cancer by age at BBD, family history, histology, and molecular marker data.

We have examined breast cancer risk by age at BBD, family history, histology, COX-2 expression, and centrosome status. We have reported on the findings in earlier sections of this report.

##### G. Analyze expression data.

Unsupervised hierarchical clustering analysis identified approximately 1240 genes that significantly differentiated the normal human mammary epithelial cell population from the rare population of mammary epithelial cells that have silenced p16 through promoter hypermethylation on the basis of expression similarities, as described above. We are pursuing appropriate candidates in the BBD samples. The first candidates being tested are COX-2, Ki67, and p16 as described in this report.

## **KEY RESEARCH ACCOMPLISHMENTS**

- We identified the degree of risk associated with the common benign epithelial entities and the extent to which age at biopsy and family history influence the risk of breast cancer in women with proliferative or atypical lesions.
- We identified a marked increased risk of breast cancer in women with three or more foci of atypia, especially for three or more foci with calcifications. Also, risk was higher in women diagnosed with atypical hyperplasia before age 45.
- We identified that a single papilloma without atypia imparts an increased risk of developing a subsequent carcinoma similar to other forms of proliferative breast disease without atypia. Atypical papilloma, particularly in the setting of multiple papillomas, imparts a breast cancer risk similar to or greater than conventional atypical ductal/lobular hyperplasias.
- We identified that the extent of lobular involution in breast tissue is an important risk indicator for the development of breast cancer. Increasing degrees of involution result in a significant reduction in breast cancer risk, even in women at “high risk” based on atypia or young age.
- We found that intense COX-2 expression is associated with a significantly greater likelihood of a subsequent breast cancer in women with atypia and represents one potential molecular target for chemoprevention strategies.
- We identified that centrosome amplification is seen more frequently in higher risk benign lesions (e.g. atypia) and is infrequently seen in non-proliferative lesions and in proliferative lesions without atypia.
- We have identified intense p16 expression as a biomarker that identifies women with a significantly greater likelihood for recurrence after lumpectomy only for DCIS. This biomarker is presently being applied to the BBD cohort.

## **REPORTABLE OUTCOMES**

### **Manuscripts**

- Hartmann LC, Sellers TA, Frost MH, Lingle WL, Degnim AC, Ghosh K, Vierkant RA, Maloney SD, Pankratz VS, Hillman DW, Suman VJ, Johnson J, Blake C, Tlsty T, Vachon CM, Melton LJ, Visscher DW. Benign breast disease and the risk of breast cancer. *New England Journal of Medicine*, 2005, 353(3):229-37.
- Lewis J, Hartmann L, Vierkant R, Maloney S, Frost M, Allers T, Visscher D. An analysis of breast cancer risk in women with single, multiple, and atypical papilloma. *Am J Surg Pathol*. 2006 Jun;30(6):665-72.

### **Manuscripts submitted**

- Degnim AC, Visscher DW, Berman H, Frost MH, Sellers TA, Vierkant RA, Maloney SD, Pankratz VS, deGroen PC, Lingle WL, Ghosh K, Penheiter L, Tlsty T, Melton LJ, Reynolds CA, Hartmann LC. Stratification of breast cancer risk in women with atypia: A Mayo cohort study, 2006.
- Milanese TR, Hartmann LC, Sellers TA, Frost MH, Vierkant RA, Maloney SD, Pankratz VS, Degnim AC, Vachon CM, Reynolds CA, Thompson RA, Melton LJ, Goode EL, Visscher DW. Age-related lobular involution and reduced risk of breast cancer, 2006.

### **Presentations**

#### **Symposium Presentations at Department of Defense Era of Hope June 9, 2005, in Philadelphia, Pennsylvania**

- “Benign Breast Disease: Evidence for Precursor Lesions” - Lynn C. Hartmann, M.D.
- “Statistical Methods to Assess the Timing and Side of Breast Cancer Relative to Benign Breast Biopsies: Implications for Potential Precursor Lesions” - V. Shane Pankratz, Ph.D.
- “Multifocal Atypia Confers Increased Risk of Breast Cancer” - Amy C. Degnim, M.D.
- “Temporal Changes in Benign Breast Disease 1967 to 1991” - Karthik Ghosh, M.D.

#### **Poster Presentations at the Department of Defense Era of Hope June 10, 2005, in Philadelphia, Pennsylvania**

- Degnim AC, Visscher D, Frost MH, Melton LJ, Vierkant RA, Maloney SC, Pankratz VS, Slleres TA, Lingle WL, Tlsty T, Berman H, Hartmann LC. “Multifocal Atypia Confers Increased Risk of Breast Cancer”
- Ghosh K, Hartmann LC, Sellers TA, Degnim AC, Pankratz VS, Blake C, Tlsty T, Melton LJ, Visscher DW. “Temporal Changes in Benign Breast Disease 1967 to 1991”
- Hartmann LC, Frost MH, Ghosh K, Degnim A, Vierkant RA, Maloney SD, Pankratz VS, Tlsty T, Blake C, Sellers TA, Lingle WL, Melton J, Visscher D “Benign Breast Disease and Breast Cancer Risk”
- Hartmann LC, Degnim A, Frost MH, Vierkant RA, Maloney SD, Sellers, TA, Pankratz VS, Tlsty T, Blake C. Lingle WL, Visscher DW. “Benign Breast Disease: Evidence for Precursor Lesions”
- Pankratz VS, Vierkant RA, Maloney SD, Degnim AC, Hartmann LC. “Statistical Methods to Assess the Timing and Side of Breast Cancer Relative to Benign Breast Biopsies: Implications for Potential Precursor Lesions”

#### **Podium Presentation at annual meeting of the United States and Canadian Academy of Pathology. February 29, 2005 in San Antonio, Texas**

- Lewis, JT, Vierkant RA, Maloney SD, Hartmann LC, Visscher DW. “Analysis of Cancer Risk among Patients with Papillary Lesions of the Breast”

#### **Podium Presentation at Society of Surgical Oncology Annual Cancer Symposium, March 3-6, 2005 in Atlanta, Georgia**

- Degnim, AC, Visscher D, Frost MH, Melton LJ, Vierkant RA, Maloney SD, Pankratz VS, Sllers TA, Lingle WL, Hartmann LC. “Multifocal Atypia Confers Increased Risk of Breast Cancer”

#### **Poster Presentation at annual meeting of American Association for Cancer Research, April 16-20, 2005 in Anaheim, California**

- Hartmann LC, Sellers TA, Frost MH, Lingle WL, Degnim AC, Ghosh K, Vierkant RA, Maloney SD, Pankratz VS, Hillman DW, Suman VJ, Johnson J, Blake C, Tlsty T, Vachon CM, Melton LJ, Visscher DW. "Benign Breast Disease and Breast Cancer Risk in the Mayo Cohort Study"

**Podium Presentation at annual meeting of American Association for Cancer Research, April 3, 2006 in Washington, D.C.**

- Hartmann LC, Lingle WL, Frost MH, Maloney SD, Vierkant RA, Pankratz VS, Tlsty T, Degnim AC, Visscher DW. "COX-2 Expression In Atypia: Correlation With Breast Cancer Risk.

## **CONCLUSIONS**

We have made significant progress on all three aims. Specifically, we have completed the cohort follow-up by questionnaires. Our pathologist has completed readings on the benign tissue for the entire cohort and has read the majority of the cancer tissues. We have evaluated the significance of the benign histologic categories (NP vs. PDWA vs. AH) and examined the risks associated with specific pathologic findings including atypia, papillomas, and involution. We calculated hazard functions for breast cancer by age at BBD and family history. We have stained the test and atypia subgroups for several immunohistochemical markers. We have identified COX-2 as an important marker of an increased risk of breast cancer in women with atypia and are currently exploring other markers. We are studying the significance of centrosome amplification in relation to subsequent breast cancer development. Additionally, we will be working closely with Wayne State to characterize the histopathology and breast cancer outcomes in a cohort of African American women with benign breast disease.

# Appendix

## **Manuscripts:**

“Benign breast disease and the risk of breast cancer”

“An analysis of breast cancer risk in women with single, multiple, and atypical papilloma”

“Breast cancer prevention strategies explored”

## **Abstracts:**

COX-2 expression in atypia: correlation with breast cancer risk

Assessment of the Gail model in a cohort of women with atypical hyperplasia

The impact of lobular involution on breast cancer risk

Analysis of cancer risk in women with radial scars of the breast

Epidemiologic comparison of disease incidence among populations: the person-years approach

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## Benign Breast Disease and the Risk of Breast Cancer

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### ABSTRACT

#### BACKGROUND

Benign breast disease is an important risk factor for breast cancer. We studied a large group of women with benign breast disease to obtain reliable estimates of this risk.

#### METHODS

We identified all women who received a diagnosis of benign breast disease at the Mayo Clinic between 1967 and 1991. Breast-cancer events were obtained from medical records and questionnaires. To estimate relative risks, we compared the number of observed breast cancers with the number expected on the basis of the rates of breast cancer in the Iowa Surveillance, Epidemiology, and End Results registry.

#### RESULTS

We followed 9087 women for a median of 15 years. The histologic findings were nonproliferative lesions in 67 percent of women, proliferative lesions without atypia in 30 percent, and atypical hyperplasia in 4 percent. To date, 707 breast cancers have developed. The relative risk of breast cancer for the cohort was 1.56 (95 percent confidence interval, 1.45 to 1.68), and this increased risk persisted for at least 25 years after biopsy. The relative risk associated with atypia was 4.24 (95 percent confidence interval, 3.26 to 5.41), as compared with a relative risk of 1.88 (95 percent confidence interval, 1.66 to 2.12) for proliferative changes without atypia and of 1.27 (95 percent confidence interval, 1.15 to 1.41) for nonproliferative lesions. The strength of the family history of breast cancer, available for 4808 women, was a risk factor that was independent of histologic findings. No increased risk was found among women with no family history and nonproliferative findings. In the first 10 years after the initial biopsy, an excess of cancers occurred in the same breast, especially in women with atypia.

#### CONCLUSIONS

Risk factors for breast cancer after the diagnosis of benign breast disease include the histologic classification of a benign breast lesion and a family history of breast cancer.

From the Divisions of Medical Oncology (L.C.H., M.H.F., J.J.), Experimental Pathology (W.L.L.), General Surgery (A.C.D.), General Internal Medicine (K.G.), Biostatistics (R.A.V., S.D.M., V.S.P., D.W.H., V.J.S.), Epidemiology (C.M.V., L.J.M.), and Anatomic Pathology (D.W.V.), Mayo Clinic College of Medicine, Rochester, Minn.; H. Lee Moffitt Cancer Center and Research Institute, Tampa, Fla. (T.A.S.); Wayne State University, Detroit (C.B.); and the University of California, San Francisco, San Francisco (T.T.). Address reprint requests to Dr. Hartmann at Mayo Clinic College of Medicine, Rochester, MN 55905.

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**B**ENIGN BREAST DISEASE IS AN IMPORTANT risk factor for a later breast cancer, which can develop in either breast.<sup>1</sup> It encompasses a spectrum of histologic entities, usually subdivided into nonproliferative lesions, proliferative lesions without atypia, and atypical hyperplasias, with an increased risk of breast cancer associated with proliferative or atypical lesions.<sup>2-4</sup> The identification of benign breast disease has become more common as the use of mammography has increased, and thus, having accurate risk estimates for women who receive this diagnosis is imperative.

Important questions remain, however, about the degree of risk associated with the common nonproliferative benign entities and the extent to which family history influences the risk of breast cancer in women with proliferative or atypical lesions. Dupont and Page found that women with nonproliferative disease did not have an increased risk of a later breast cancer.<sup>2</sup> By contrast, a companion study to the National Surgical Adjuvant Breast and Bowel Project (NSABP) Breast Cancer Prevention Trial (P1) found a relative risk of 1.6 for women who received a diagnosis of a "lower category" of benign breast disease.<sup>5</sup> A limitation of the NSABP study, however, was the lack of central pathological review.

Another major question concerns the possible interplay between atypia and a family history of breast cancer. The Dupont and Page study found that women with atypia and a family history had 11 times the risk of those with nonproliferative lesions and no family history.<sup>2</sup> However, two other major studies of benign breast disease<sup>6,7</sup> did not find a significant interaction between atypia and family history. The duration of increased risk after a finding of benign disease on biopsy is also uncertain.<sup>2,4,8</sup>

Studies of benign breast disease can also clarify whether there is a continuum of breast alterations that culminates in breast cancer. However, it remains unclear which of the benign entities are actual precursors and which reflect a background of increased risk involving all breast tissue in a woman. Determining the extent of agreement between the side (right or left) of the benign lesion and the subsequent breast cancer is one means of assessing these issues.

To investigate these questions, we studied 9087 women with benign breast disease for whom we had follow-up data on breast-cancer events. This cohort has been followed for a median of 15 years, and 707 breast cancers have developed, making this, to our knowledge, one of the largest such studies of its

kind. We report on the risk of breast cancer according to histologic findings, the age at diagnosis of benign breast disease, and the strength of the family history. We also recorded the side of the cancer (ipsilateral or contralateral) and the time to the diagnosis of cancer.

## METHODS

### STUDY POPULATION

We accessed data from the Mayo Clinic Surgical Index and Pathology Index to identify all women 18 to 85 years of age who had undergone surgical excision of a benign breast lesion during the 25-year period from January 1, 1967, through December 31, 1991. For women who had more than one biopsy during this period, we used the first sample. The original list contained 12,132 women, but we excluded 1,047 women for any of the following: a diagnosis of breast cancer or lobular carcinoma in situ at, before, or within six months after the biopsy of the benign lesion; mastectomy (unilateral or bilateral) or breast reduction at or before biopsy; or refusal to allow use of their medical records for research.<sup>9</sup> This left 11,085 women. Of these, 1053 (9.5 percent) had no follow-up information after the biopsy. Thus, a total of 10,032 women met our criteria for study entry and had follow-up information. Of these, 945 women had unusable or unavailable biopsy specimens of the benign lesion. The remaining group of 9087 women constitutes our study cohort. The relative risks of breast cancer (described below) did not differ significantly between the 10,032 women who met our criteria and the 9087 women who made up the study cohort (1.59 and 1.56, respectively).

### FAMILY HISTORY AND FOLLOW-UP

A questionnaire designed for this study was used to obtain information about family history and other possible risk factors for breast cancer. Thus, our family-history data were obtained at the time of follow-up contact. We categorized family history as none, weak, or strong. The criteria for a strong family history were as follows: at least one first-degree relative with breast cancer before the age of 50 years or two or more relatives with breast cancer, with at least one being a first-degree relative. Any lesser degree of family history of breast cancer was categorized as weak. The questionnaire also asked about breast-cancer occurrences. Follow-up for breast-cancer events was also obtained through the comprehensive (inpatient and outpatient) Mayo medical



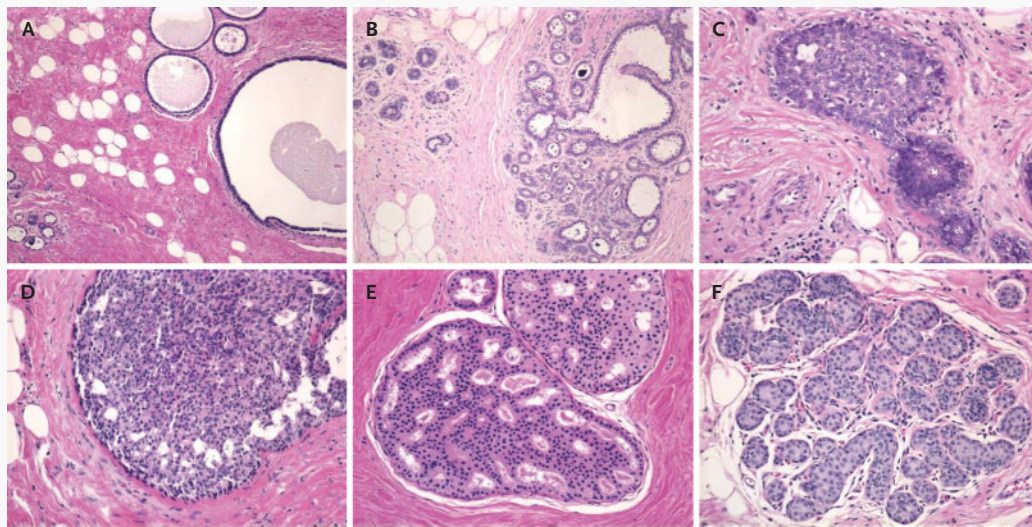
record. Questionnaire information was available for 5619 women (61.8 percent). Of the questionnaires, 604 (10.7 percent) were completed by proxy (the next of kin of a deceased patient). As of August 1, 2004, 7260 (79.9 percent) members of the cohort were still alive. All protocol procedures and patient-contact materials were reviewed and approved by the institutional review board of the Mayo Clinic; returning the contact materials was considered implied consent.

#### HISTOLOGY

Stored hematoxylin-and-eosin-stained sections from each participant were evaluated by a breast pathologist who was unaware of the initial histologic diagnoses and patient outcomes. Biopsy findings were classified according to the criteria of Page et al.<sup>2,10</sup> into the following categories: nonproliferative fibrocystic changes, proliferative fibrocystic changes without atypia, and proliferative fibrocystic changes with atypia (atypical ductal hyperplasia, atypical lobular hyperplasia, or both) (Fig. 1).<sup>2,10</sup> Biopsy specimens were designated as having proliferative fibrocystic changes if they contained any of the following: ductal hyperplasia (greater than mild), papilloma, radial scar, or sclerosing adenosis. Cysts, fibroadenoma, or columnar changes were considered nonproliferative unless they also contained one of the lesions denoted above.

#### STATISTICAL ANALYSIS

The duration of follow-up was calculated as the number of days from biopsy of the benign lesion to the date of the diagnosis of breast cancer, death, or last contact. We estimated relative risks on the basis of standardized incidence ratios (SIRs), dividing the observed numbers of incident breast cancers by population-based expected counts. We calculated these expected counts by apportioning each woman's follow-up into five-year age and calendar-



**Figure 1. Histopathological Appearance of Benign Breast Disease (Hematoxylin and Eosin).**

Panel A shows nonproliferative fibrocystic changes: the architecture of the terminal-duct lobular unit is distorted by the formation of microcysts, associated with interlobular fibrosis. Panel B shows proliferative hyperplasia without atypia. This is adenosis, a distinctive form of hyperplasia characterized by the proliferation of lobular acini, forming crowded gland-like structures. For comparison, a normal lobule is on the left side. Panel C also shows proliferative hyperplasia without atypia. This is moderate ductal hyperplasia, which is characterized by a duct that is partially distended by hyperplastic epithelium within the lumen. Panel D again shows proliferative hyperplasia without atypia, but this is florid ductal hyperplasia: the involved duct is greatly expanded by a crowded, jumbled-appearing epithelial proliferation. Panel E shows atypical ductal hyperplasia: these proliferations are characterized by a combination of architectural complexity with partially formed secondary lumens and mild nuclear hyperchromasia in the epithelial-cell population. Panel F shows atypical lobular hyperplasia: monotonous cells fill the lumens of partially distended acini in this terminal-duct lobular unit.



period categories, thereby accounting for differences associated with these variables. We used the Iowa Surveillance, Epidemiology, and End Results (SEER) registry as the reference population because of its demographic similarities to the Mayo Clinic population (80 percent of cohort members reside in the upper Midwest). Over 95 percent of our cohort was white, equivalent to that reported in Iowa census data during the study period.<sup>11</sup> In the SIR analyses, we considered the time since the original biopsy as a time-dependent variable and all other factors as fixed.

Associations between the risk of breast cancer and histologic findings, the age at diagnosis of be-

nign breast disease, and the strength of the family history of cancer, as well as pairwise combinations of these variables, were examined with the use of Cox proportional-hazards regression analysis. The main effects for each categorized variable and the corresponding interaction terms were included in each model, and the statistical significance of each interaction was evaluated with the use of a multiple-degree-of-freedom likelihood-ratio test.

We studied ipsilateral and contralateral breast cancer as a function of the time since biopsy by estimating the relative risk of cancer in the same as compared with the opposite breast for five-year intervals. When calculating the incidence of ipsilat-

**Table 1. Characteristics of the Women According to the Histologic Category of Benign Breast Disease.\***

Characteristic	All Women (N=9087)	Nonproliferative Disease (N=6061)	Proliferative Disease without Atypia (N=2690)	Atypical Hyperplasia (N=336)
Percentage of total	100.0	66.7	29.6	3.7
Age at biopsy — no. of women (%)				
<40 yr	1841 (20.3)	1500 (24.7)	323 (12.0)	18 (5.4)
40–49 yr	2474 (27.2)	1621 (26.7)	770 (28.6)	83 (24.7)
50–59 yr	2145 (23.6)	1297 (21.4)	759 (28.2)	89 (26.5)
60–69 yr	1639 (18.0)	1034 (17.1)	522 (19.4)	83 (24.7)
≥70 yr	988 (10.9)	609 (10.0)	316 (11.7)	63 (18.8)
Mean age at biopsy — yr	51.4±14.3	49.9±14.8	53.9±12.6	57.8±12.3
Menopausal status at biopsy — no. of women (%)†				
Premenopausal (<45 yr)	2948 (32.4)	2246 (37.1)	652 (24.2)	50 (14.9)
Perimenopausal (45–55 yr)	2583 (28.4)	1610 (26.6)	871 (32.4)	102 (30.4)
Postmenopausal (>55 yr)	3556 (39.1)	2205 (36.4)	1167 (43.4)	184 (54.8)
Family history of breast cancer — no. of women (%)				
Unknown	4279 (47.1)	2970 (49.0)	1170 (43.5)	139 (41.4)
Known	4808 (52.9)	3091 (51.0)	1520 (56.5)	197 (58.6)
None	2668 (55.5)	1735 (56.1)	831 (54.7)	102 (51.8)
Weak	1174 (24.4)	756 (24.5)	378 (24.9)	40 (20.3)
Strong	966 (20.1)	600 (19.4)	311 (20.5)	55 (27.9)
Breast-cancer status as of August 2004 — no. of women (%)				
Negative	8380 (92.2)	5682 (93.7)	2426 (90.2)	272 (81.0)
Positive	707 (7.8)	379 (6.3)	264 (9.8)	64 (19.0)
Vital status — no. of women (%)				
Deceased	1827 (20.1)	1172 (19.3)	566 (21.0)	89 (26.5)
Alive	7260 (79.9)	4889 (80.7)	2124 (79.0)	247 (73.5)

\* Plus-minus values are means ±SD.

† Menopausal status was categorized according to the age at breast biopsy.

eral cancer, we censored follow-up on women with contralateral cancer after the date of diagnosis. Similarly, when calculating the incidence of contralateral cancer, we censored follow-up on women with ipsilateral cancer after the date of diagnosis. Data on women missing information on the side of the cancer or women who had bilateral biopsies or cancer were not included in these analyses. This approach yields identical numbers of person-years for each type of event. As a result, the length of follow-up is no longer a factor in the analysis and the relative risks are equivalent to simple ratios of event counts. We therefore used properties of the binomial distribution to obtain exact P values and 95 percent confidence intervals for these relative risks.<sup>12</sup> Statistical tests were two-sided, and analyses were conducted with the use of SAS (SAS) and Splus (In-sightful) software.

## RESULTS

## CHARACTERISTICS OF PATIENTS AND PATHOLOGICAL SPECIMENS

The final cohort consisted of 9087 women with benign breast disease as determined by open surgical biopsy. Table 1 shows the age at the time of the biopsy, likely menopausal status on the basis of age, and the strength of the family history of breast cancer according to the histologic findings for the benign lesion. The broad histologic classifications included nonproliferative disease in 6061 (66.7 percent), proliferative disease without atypia in 2690 (29.6 percent), and atypical hyperplasia in 336 (3.7 percent). Figure 1 shows examples of these lesions. The mean age was 51.4 years, but women with nonproliferative findings were slightly younger, whereas those with atypia tended to be older (mean age,

**Table 2. Risk Factors for Breast Cancer after the Diagnosis of Benign Breast Disease.\***

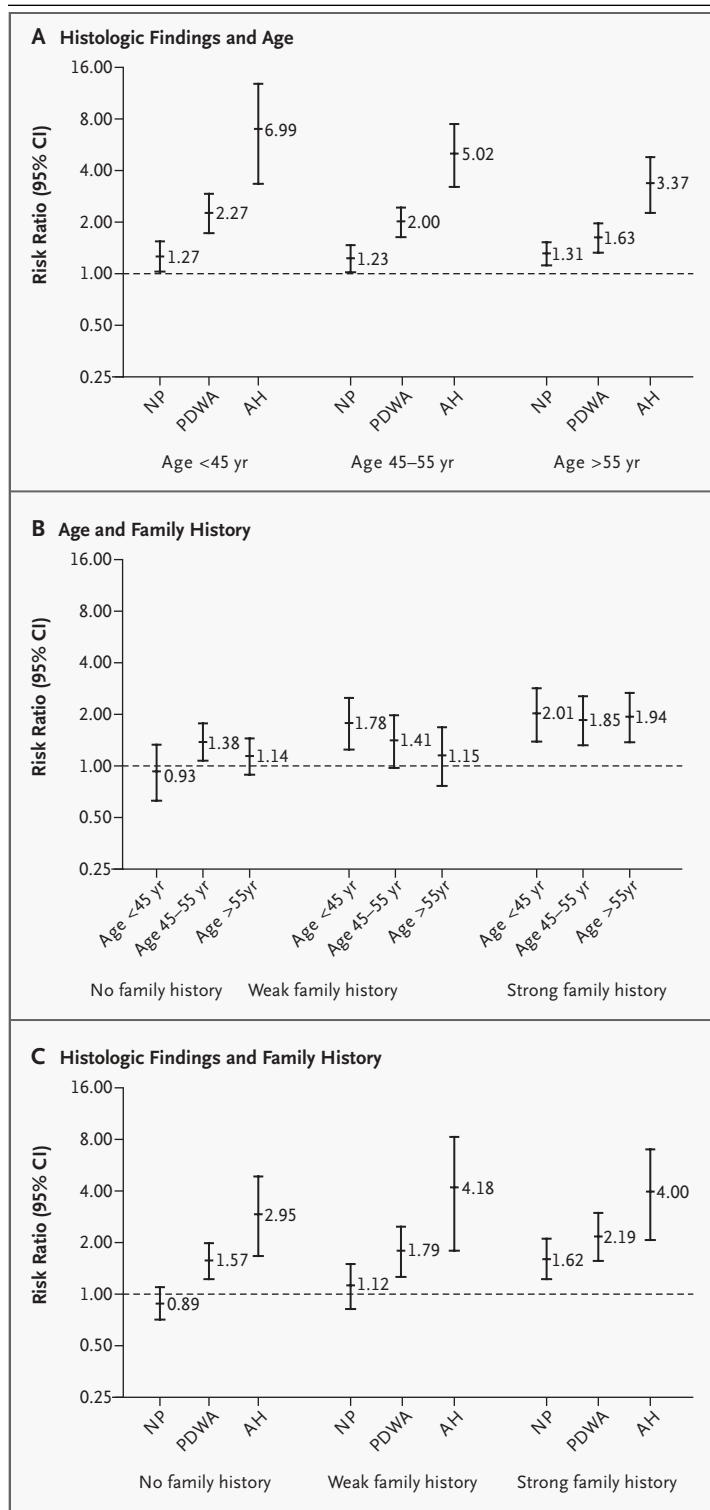
Characteristic	No. of Women	Person-Years	No. of Observed Events	No. of Expected Events	Relative Risk (95% CI)†
Overall	9087	144,881	707	453.0	1.56 (1.45–1.68)
Age at diagnosis of benign breast disease					
<30 yr	726	13,593	21	11.5	1.83 (1.13–2.80)
30–39 yr	1115	20,169	71	38.3	1.85 (1.45–2.34)
40–49 yr	2474	45,780	212	136.3	1.56 (1.35–1.78)
50–59 yr	2145	34,100	196	125.9	1.56 (1.35–1.79)
60–69 yr	1639	21,364	142	94.5	1.50 (1.27–1.77)
≥70 yr	988	9,874	65	46.6	1.40 (1.08–1.78)
Menopausal status‡					
Premenopausal (age <45 yr)	2948	54,419	169	106.1	1.59 (1.36–1.85)
Perimenopausal (age 45–55 yr)	2583	45,872	245	153.4	1.60 (1.40–1.81)
Postmenopausal (age >55 yr)	3556	44,590	293	193.6	1.51 (1.35–1.70)
Histologic findings					
Nonproliferative disease	6061	99,109	379	297.7	1.27 (1.15–1.41)
Proliferative disease without atypia	2690	41,610	264	140.2	1.88 (1.66–2.12)
Atypical hyperplasia	336	4,161	64	15.1	4.24 (3.26–5.41)
Family history of breast cancer§					
None	2668	44,974	171	145.4	1.18 (1.01–1.37)
Weak	1174	21,472	94	65.9	1.43 (1.15–1.75)
Strong	966	18,087	110	57.0	1.93 (1.58–2.32)

\* Numbers of women, person-years, and events may not sum to overall totals because of rounding.

† The relative risk reflects the observed number of events as compared with the number expected on the basis of Iowa SEER data. All analyses account for the effects of age and calendar period. CI denotes confidence interval.

‡ Menopausal status was categorized according to the age at breast biopsy.

§ Information on family history was available for 4808 of the 9087 women.



**Figure 2. Risk-Factor Interaction Profiles for Benign Breast Disease, Comparing the Number of Events Observed with the Number Expected.**

Expected events account for age and calendar period and are calculated with the use of Iowa SEER rates. CI denotes confidence interval, NP nonproliferative disease, PDWA proliferative disease without atypia, and AH atypical hyperplasia.

weakly positive in 1174 (24.4 percent), and strongly positive in 966 (20.1 percent). More women with atypia than without atypia had a strong family history of breast cancer (27.9 percent vs. 19.8 percent,  $P=0.06$ ). The risk of cancer was highest in the group with atypia: breast cancer developed in 64 of the 336 women (19.0 percent).

#### FEATURES OF BENIGN BREAST DISEASE AND SUBSEQUENT RISK OF BREAST CANCER

Patients in the cohort were followed for a median of 15 years. A total of 1827 women (20.1 percent) had died and 7260 (79.9 percent) were alive as of August 2004. We have documented 707 breast cancers to date. The median time from the original biopsy to the diagnosis of breast cancer was 10.7 years. Table 2 shows the estimated relative risks of breast cancer associated with the age at the initial biopsy, the strength of the family history, menopausal status, and histologic findings of the biopsy, as compared with expected population-based incidence. The estimated relative risk of breast cancer in the cohort was 1.56 (95 percent confidence interval, 1.45 to 1.68). The risk was inversely associated with the age at biopsy, with younger women having a greater risk than older women. The type of benign breast disease identified at biopsy was a major predictor of risk. Atypical hyperplasia had a relative risk of 4.24 (95 percent confidence interval, 3.26 to 5.41), proliferative disease without atypia had a relative risk of 1.88 (95 percent confidence interval, 1.66 to 2.12), and nonproliferative lesions had a relative risk of 1.27 (95 percent confidence interval, 1.15 to 1.41). Family history was an independent risk factor. For women with no known family history of breast cancer, the relative risk was only 1.18 (95 percent confidence interval, 1.01 to 1.37), as compared with 1.43 (95 percent confidence interval, 1.15 to 1.75) for women with a weak family history and 1.93 (95 percent confidence interval, 1.58 to 2.32) for those with a strong family history.

49.9 and 57.8 years, respectively;  $P<0.001$ ). Information on family history was available for 4808 women and was negative in 2668 (55.5 percent),

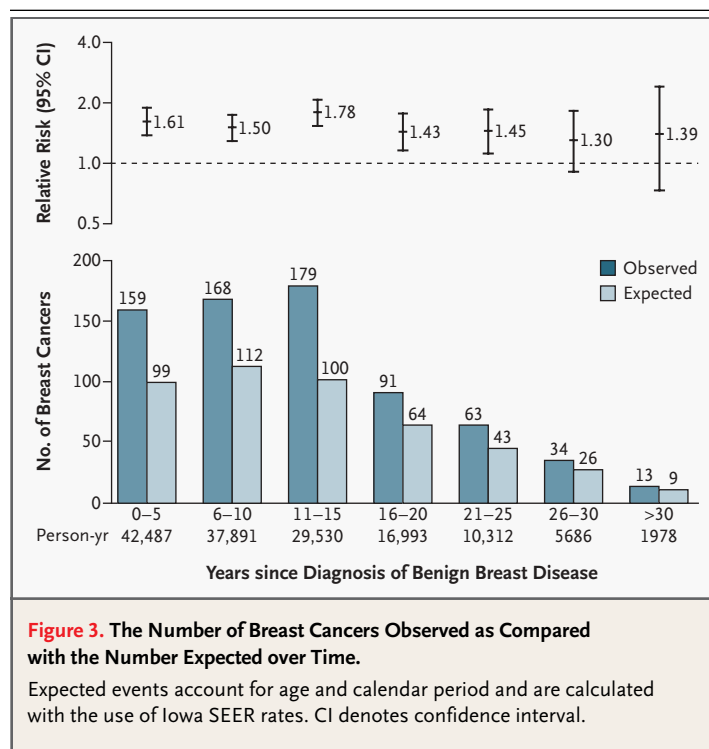
Figure 2 shows possible interactions between pairs of the major risk factors of age, histologic findings, and family history. No significant interactions were observed between age and family history or between histologic findings and family history, including atypia and family history. However, there was a significant interaction between age and histologic findings ( $P=0.05$ ): the risk of breast cancer was 6.99 times the expected risk among women who received a diagnosis of atypia before the age of 45 years; the risk was 5.02 times the expected risk when the atypia was diagnosed between the ages of 45 and 55 years and 3.37 times the expected risk when it was diagnosed after the age of 55 years. An important finding was that for women with non-proliferative disease and no family history or a weak family history, there was no increase in the risk of breast cancer.

#### TIME COURSE AND SIDE OF BREAST CANCER AFTER BENIGN BREAST DISEASE

Figure 3 shows the observed and expected numbers of cancers at five-year intervals. The excess risk persisted for at least 25 years after the initial biopsy and perhaps for 30 years or more, but accuracy was low after 25 years. Figure 4 shows a further breakdown of breast cancers into ipsilateral or contralateral according to the histologic findings in the benign lesion. Of the 616 unilateral cancers, 342 (55.5 percent) developed in the same breast as the initial biopsy and 274 (44.5 percent) developed in the contralateral breast. In the remaining 91 cases, there were bilateral events, either benign or malignant, or information on the side of the cancer was missing. During the first 10 years, there was an excess of ipsilateral cancers, with relative risks of ipsilateral as compared with contralateral cancer of 1.88 (95 percent confidence interval, 1.33 to 2.64) for years 0 through 5 and 1.34 (95 percent confidence interval, 0.96 to 1.85) for years 6 through 10. The 35 women with atypia in whom breast cancer developed within 10 years after the initial biopsy were 2.5 times as likely ( $P=0.02$ ) to have the cancer in the same breast as in the opposite breast.

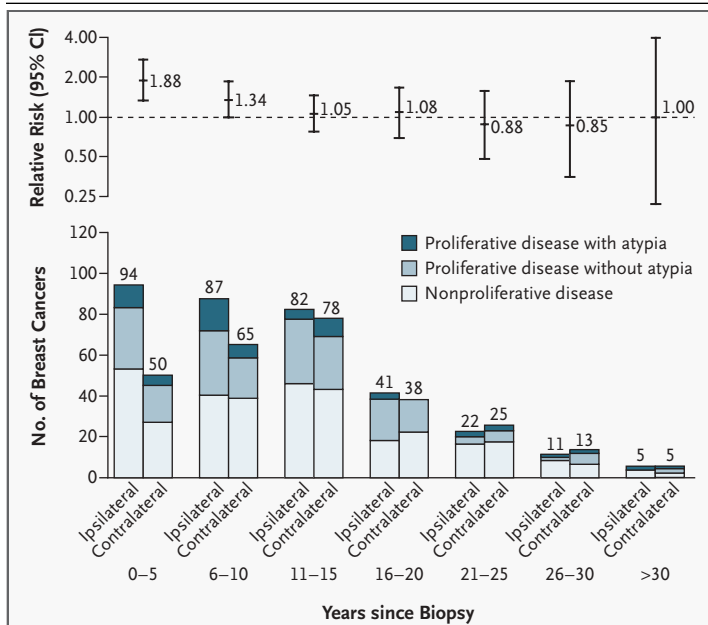
#### DISCUSSION

Retrospective and prospective studies have shown a relative risk of breast cancer of 1.5 to 1.6 for women with benign breast disease as compared with women in the general population.<sup>2,5-7,13-21</sup> The histologic appearance of the benign lesion is a major



determinant of risk, yet not all large studies have had access to tissue for re-review. Our investigation was based on a single-institution resource with long-term and complete follow-up for cancer events. All samples containing the benign lesion were read by a breast pathologist who applied current histologic classifications. More than 700 breast cancers developed in this cohort, giving our study good statistical power. The relative risk of breast cancer for our cohort overall was 1.56 (95 percent confidence interval, 1.45 to 1.68), and this increased risk persisted for at least 25 years after the initial biopsy.

The histologic appearance of the benign lesion is strongly associated with the risk of breast cancer. For biopsies with nonproliferative findings, the relative risk was 1.27 (95 percent confidence interval, 1.15 to 1.41), as compared with a relative risk of 1.88 (95 percent confidence interval, 1.66 to 2.12) for findings of proliferative changes but no atypia and of 4.24 (95 percent confidence interval, 3.26 to 5.41) for a finding of atypical hyperplasia. When the family history is known, risk profiles can be refined. For women with nonproliferative findings and no family history or a weak family history of breast cancer, we observed no increased risk. This finding is important, because a sizable proportion



**Figure 4. Comparison of the Number of Ipsilateral Breast Cancers with the Number of Contralateral Breast Cancers over Time, According to the Histologic Appearance of Benign Breast Disease.**

Results are shown for 616 cancers (342 ipsilateral and 274 contralateral cancers). The remaining 91 cases include women with bilateral benign or malignant lesions or for whom the side of the benign or malignant lesion was unknown. CI denotes confidence interval.

of women with benign breast disease are in this group (52 percent of our cohort with a known family-history status). Dupont and Page made a similar observation in their 1985 report.<sup>2</sup> However, a recent NSABP study found a significantly increased risk of breast cancer among women with lower-category benign breast disease, including nonproliferative disease.<sup>5</sup> In the NSABP P1 trial, which included more than 13,000 women, 1376 had a breast biopsy with benign findings over a mean follow-up period of 79 months. Breast cancer developed in 47 of these women. On the basis of pathology reports from contributing centers, the investigators reported a relative risk of 1.6 among women with lower category findings on breast biopsy as compared with P1 participants who did not undergo a breast biopsy.<sup>5</sup>

In our study, the degree of family history was an independent risk factor. In women with a strong family history of breast cancer, even nonproliferative findings were associated with a risk ratio of 1.62. This subgroup may parallel the high-risk NSABP cohort.<sup>5</sup> Women with atypia are at significantly in-

creased risk, but a family history did not significantly modify the atypia-associated risk (Fig. 2). The risk was four times the expected risk among women with atypia and a family history of breast cancer, regardless of the degree of their family history; among women with atypia without a family history of breast cancer, the risk ratio was 2.95 (95 percent confidence interval, 1.65 to 4.87).

The age at the diagnosis of benign breast disease appears to modify the risks related to the histologic appearance of benign breast disease. The presence of atypia in women under 45 years of age conveyed twice the risk observed among women over 55 years of age (6.99 and 3.37, respectively), which might relate, in part, to menopausal status. The Breast Cancer Detection and Demonstration Project showed that the risk of breast cancer among premenopausal women with atypia was elevated by a factor of 12.0 (95 percent confidence interval, 2.0 to 68.0), as compared with 3.3 among postmenopausal women with atypia (95 percent confidence interval, 1.1 to 10.0), but the numbers of patients in the study were small.<sup>22</sup> The Nurses Health Study also showed an increased risk of breast cancer among premenopausal women with atypia.<sup>7</sup> However, in the NSABP study of women with lower categories of benign breast disease, the risk of breast cancer was greatest among postmenopausal women.<sup>5</sup>

Understanding the risk associated with benign breast disease is important because the increasing use of mammography has increased the frequency of breast biopsies, most of which yield benign findings. In a retrospective study of women undergoing annual mammographic screening, Elmore et al. found that 18.6 percent of women underwent a biopsy after 10 screening mammograms.<sup>23</sup> The use of hormone therapy may also affect the frequency of breast biopsies. Chlebowski et al., reporting for the Women's Health Initiative investigators, found that relatively short-term therapy with estrogen plus progestin increased the percentage of women with abnormal mammograms, a major indicator for breast biopsy.<sup>24</sup>

Regarding the possibility of malignant precursors within benign breast disease, we have information on the side and the time to breast cancer for 616 unilateral events. An excess of breast cancers occurred in the same breast during the first years of follow-up, especially in women with atypia (Fig. 4). This finding suggests that precursors to breast cancer exist in benign breast disease. Work in model systems of early steps in mammary carcinogenesis



has identified alterations in key regulatory indicators that can be studied in selected benign breast lesions.<sup>25,26</sup>

In summary, our study shows that histologic features, the age at biopsy, and the degree of family history are major determinants of the risk of breast cancer after the diagnosis of benign breast disease. We found no increased risk among women with nonproliferative lesions, unless a strong family history was present. No significant interaction between atypia and family history was apparent. The excess

risk of cancer in the ipsilateral breast in the first 10 years after the diagnosis of benign breast disease, especially in women with atypia, points to the presence of precursors in some women.

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# An Analysis of Breast Cancer Risk in Women With Single, Multiple, and Atypical Papilloma

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**Abstract:** Breast papillomas may be single or multiple and associated with atypical ductal or lobular hyperplasias (ADH/ALH). The risk of breast carcinoma development in patients with papillomas, particularly those with multiple or atypical lesions, is incompletely defined. Fibrocystic lesions were histopathologically classified in a benign breast disease cohort of 9155 who underwent biopsy from 1967 to 1991, with papilloma assessment in 9108. Individuals with papillomas (N = 480) were classified into 4 groups: single papilloma (SP, N = 372), single papilloma with ADH or ALH (SP+A, N = 54), multiple (> 5) papillomas (MP, N = 41), and multiple papillomas with ADH or ALH (MP+A, N = 13). Those without papillomas were classified as nonproliferative (NP, N = 6053), proliferative without atypia (PDWA, N = 2308), and ADH/ALH [atypical hyperplasia (AH), N = 267]. The relative risk of cancer development within our cohort was compared to that expected in the general population using standardized incidence ratios. The relative risk of breast cancer development associated with SP [2.04, 95% confidence interval (CI) 1.43-2.81] was greater than NP (1.28, 95% CI 1.16-1.42) but similar to PDWA (1.90, 95% CI 1.66-2.16). The risk associated with SP+A (5.11, 95% CI 2.64-8.92) was highly elevated but not substantively different than atypical hyperplasia (4.17, 95% CI 3.10-5.50). Patients with MP are at increased risk compared with PDWA or SP (3.01, 95% CI 1.10-6.55), particularly those with MP+A (7.01, 95% CI 1.91-17.97). There was a marginal increase in breast cancer risk (16%) among patients with proliferative disease if a papilloma was present, but this did not reach statistical significance ( $P = 0.29$ ). The observed frequency of ipsilateral (vs. contralateral) breast cancer development in papilloma subsets was not significantly different than other patient groups. We conclude that SP imparts a cancer risk similar to conventional proliferative fibrocystic change. The presence of papilloma in, or associated with, atypia does not modify the risk connotation of ADH/ALH overall. MP constitutes a proliferative breast disease subset having unique clinical and biologic behavior.

**Key Words:** intraductal papilloma, atypical hyperplasia, breast cancer

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Papillomas of the breast are defined by a constellation of pathologic findings including: (1) a discrete intraductal polypoid lesion with, (2) an arborizing fibrovascular stroma covered by a layer of myoepithelium, and (3) a second layer of columnar or cuboidal epithelium. They often form palpable nodules, reaching considerable size in some cases, although many are microscopic.<sup>4,5,12,13</sup> They are informally classified by anatomic location: central/subareolar papillomas are usually single but may reach considerable size and become symptomatic. Peripheral lesions, in contrast, are generally smaller but may be multiple and recurrent.<sup>4</sup> Papillomas are often accompanied by significant epithelial hyperplasia and/or periductal sclerosis, resulting in microscopically complex lesions.<sup>1,11,25</sup> Atypical hyperplasia (AH) may also be present within or adjacent to papilloma.<sup>19,25</sup> In these so-called atypical papillomas, the histologic distinction from ductal carcinoma in situ may be extremely problematic.

Most early investigators considered intraductal papillomas to be benign lesions without malignant potential or implied risk of developing a subsequent carcinoma.<sup>13,14</sup> More recent studies have demonstrated that these lesions, like other forms of proliferative breast disease, do increase the risk of developing carcinoma.<sup>3,6,8,9,11,25</sup> Some have suggested that papillomas may behave as direct precursor lesions.<sup>7,27</sup> Neither view, however, has been empirically tested in a sufficiently large cohort of patients with long term follow-up and appropriate population controls. Further, most studies which specifically address pathologic subsets thought to be biologically more aggressive, such as multiple papilloma or atypical papilloma, consist of relatively small numbers or are enriched by selective inclusion of cases derived from referral consult practices.<sup>19,25,28</sup>

We have recently completed pathologic evaluation of a benign breast disease cohort, consisting of all open benign breast biopsies performed at the Mayo Clinic between 1967 and 1991 (N = 9155).<sup>15</sup> All papillary lesions present in these biopsies were routinely defined as a

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component of our microscopic examination. Our objective in this study is to survey the incidence, histologic patterns and relative cancer risk associated with benign papillomas of the breast. We will specifically address the significance of multiple papillomas and papillomas with atypia and whether there is evidence to suggest they are direct precursors.

## MATERIALS AND METHODS

### Patient Selection

Patients with benign diagnoses on open excisional biopsy (OEB) of the breast, performed between January 1, 1967 and December 31, 1991, constituted the study population. Searching the Mayo Clinic Surgical Index and Pathology Index identified the cases. The details of the derivation of the study cohort have been published previously.<sup>15</sup> Briefly, subjects were excluded from the study if they met one or more of the following criteria: (1) cancer diagnosis before, at, or within 6 months of the OEB (accounting for possible occult malignancy), (2) unilateral or bilateral mastectomy or reduction before OEB, (3) refusal of research authorization, (4) no follow-up information available, or (5) slides unavailable. Of the 9155 women who met the study criteria, papilloma information for 47 were unavailable. The resulting 9108 patients constituted the study cohort, with a mean follow-up of 16 years.

### Pathology Review

A pathologist with expertise in breast pathology (D.V.) reviewed the original hematoxylin and eosin (H&E) stained slides of all cases without knowledge of original diagnosis or subsequent outcome. Cases were classified into one of three general categories: nonproliferative (NP) fibrocystic changes, proliferative changes without atypia (PDWA), and AH. NP fibrocystic changes included cyst formation, stromal fibrosis, apocrine metaplasia, and noncomplex fibroadenoma. Proliferative changes without atypia included ductal hyperplasia of usual type, sclerosing adenosis, radial scars/complex sclerosing lesions, and papilloma(s). Atypical ductal or lobular hyperplasia (ADH/ALH) constituted the AH category and the presence or absence of each was documented in each case. The criteria for classification as AH (see below) were applied to epithelial proliferations within or outside of the papilloma.

A papilloma was defined as an intraductal epithelial proliferation of any size that is supported by branching fibrovascular stalks that contains myoepithelium. In many cases they were not the predominant lesions in the biopsy specimen (eg, florid duct hyperplasia with an incidental, microscopic papilloma). Papillomas were classified as solitary (SP) or multiple (MP), the latter defined as a papillary lesion containing at least 5 papillomas in 2 nonconsecutive tissue blocks.<sup>1</sup> The presence or absence of AH (ADH and/or ALH) was also documented in the papilloma cases. If ADH or ALH was identified within the papilloma or in the surrounding

parenchyma, then the case was classified as a single papilloma with atypia (SP + A) or multiple papillomas with atypia (MP + A). For the SP + A cases, the location of the atypia (inside and/or outside the papilloma) was recorded.

ADH was defined according to the criteria of Page.<sup>23-25</sup> These lesions exhibited architecturally complex cribriformlike proliferations of monotonous cells that lacked malignant cytologic features and were confined to an area measuring < 3 mm in greatest dimension. Within papillomas, these atypical lesions only partially involved a "basement membrane bound space," with a second nonatypical population of cells composing the remainder.<sup>24</sup> ALH was defined as a proliferation of polygonal, evenly spaced cells with round, monotonous nuclei, and scant cytoplasm.<sup>23</sup> ALH was characterized by partial expansion of acini by atypical cells, often with preservation of luminal spaces, involving less than half of the acini in a lobule.

### Statistical Analysis

Data were descriptively summarized using frequencies and percentages for categorical variables, and means and standard deviations for continuous variables. We formally compared distributions of certain attributes across papilloma-defined subgroups using *t* tests and analyses of variance for the continuous variables and  $\chi^2$  tests for categorical variables.

The length of the follow-up for each woman in the study was calculated as the number of days (followed by division by 365.25 to calculate years) from her benign biopsy to the date of breast cancer diagnosis, date of death, or date of last contact. The cumulative incidence of breast cancer by papilloma status was estimated using Kaplan-Meier curves. We compared the observed number of incident breast cancer events in our cohort, stratified by papilloma status, to that expected in the general population using standardized incidence ratios (SIRs). Each individual's person years were apportioned into 5-year age and calendar period categories. Overall category-specific follow-up was then multiplied by the corresponding age-stratified and calendar period-stratified surveillance epidemiology and end results (SEER) incidence rates, and these results were then summed across all categories to calculate the expected number of events. Thus, all risk ratios account for the potentially confounding effects of age and calendar period. The Iowa SEER registry was used as the primary standard population, due to both the proximity of its participants to the Mayo Clinic catchment area and racial/ethnic similarities to our cohort.

As proliferative disease is a complex mixture of many different attributes which may synergistically affect the risk of breast cancer, it is possible that other forms of proliferative change could confound the association of papillomas and breast cancer. Thus, we sought to assess the independent modifying effects of different forms of proliferation using Poisson regression analyses, modeling the individual-specific, log-transformed expected event



rate as the offset term. This approach facilitates the calculation of SIRs with the added flexibility that generalized linear models provide, such as covariate adjustment and formal assessment of heterogeneity. The Iowa SEER registry was again used to calculate the expected event rates. Analyses were subset to women with proliferative disease. Based on the Poisson models, we calculated relative SIRs (rSIRs), directly comparing ratios of SIRs across levels of each of the proliferation attributes. The accompanying *P* values assess the heterogeneity of breast cancer SIRs across levels of the attribute. The following types of proliferation were examined: presence of atypia, presence of papillomas, presence of sclerosing adenosis, and presence of radial scars. Two sets of poisson models were fit: one that accounted only for the effects of age and calendar period, and one that accounted additionally and simultaneously for the effects of the other proliferation attributes.

We compared the potentially differential risk of ipsilateral versus contralateral breast cancer within the cohort across papilloma-defined subgroups using a competing risk approach, based on the Poisson distribution. Women with missing biopsy or cancer side information, or with benign breast disease (BBD) or cancer diagnosed bilaterally, were excluded from these analyses. For each subgroup, we compared the incidence rate for ipsilateral cancer to the corresponding rate for contralateral cancer. When calculating incidence for ipsilateral cancer, individuals with contralateral cancer were censored at their date of diagnosis, and vice versa. This approach yields identical person years for each event type, reducing comparisons of incidence to simple comparisons of counts via  $\chi^2$  tests of significance. All statistical tests were 2-sided, and all analyses were carried out using the SAS software system (SAS Institute, Inc, Cary, NC).

## RESULTS

### Pathologic Findings

The Mayo benign breast disease cohort has been presented in detail elsewhere.<sup>15</sup> Our current cohort includes 9108 patient biopsies, classified as follows: 6053 (66.5%) NP; 2308 (25.3%) PDWA; and 267 (2.9%) AH. Papilloma was identified in 480 (5.3%) of the biopsies,

distributed within the PDWA and AH categories. The majority of papillomas (372, 4.1%) were SP. There were 54 SP + A (0.6%), 41 MP (0.5%), and 13 (0.1%) MP + A. The mean age at biopsy was youngest for the NP group (49.9, SD 14.8), intermediate for the nonatypical proliferative groups [PDWA 53.6 (SD 12.1), SP 55.2 (SD 14.5) and MP 53.9 (SD 15.5)], and oldest for the atypical groups [AH 57.3 (SD 11.6), SP + A 59.1 (SD 13.4), and MP + A 65.1 (SD 14.0)].

Because family history is a known risk factor for the development of carcinoma, we compared the frequency of papilloma status with family history to determine if there were any differences among the subsets. Family history was available in 4846 (53%) of the 9108 cases. As Table 1 illustrates, the majority of cases in all subsets did not have a family history of breast cancer. The remainder of the cases varied from a weak to strong family history.  $\chi^2$  tests revealed no differences in distribution of papilloma(s) across levels of family history (*P* = 0.49).

Papillomas were accompanied by a complex mixture of proliferative changes. Sclerosing adenosis and usual ductal hyperplasia were both present in at least 50% of cases from each papilloma subgroup (Table 2). Radial scars also occurred at significantly increased frequency (16% SP, 33% SP + A, 34% MP, 31% MP + A) compared to individuals without papillomas (4%, *P* < 0.001). Among papilloma cases, radial scars were significantly more common in SP + A, MP, and MP + A compared with SP (*P* < 0.001,  $\chi^2$  test).

With respect to atypia in the setting of papilloma, most cases (33/51, 65%) contained ADH alone. There were 6 (12%) with ALH and 12 with both ADH and ALH (23%). Examples of atypical papilloma are illustrated in Figures 1 to 4. Of the 45 cases with ADH, atypia was present within the papilloma in 16 (36%), outside of the papilloma in 17 (38%), and present both inside and outside the papilloma in 12 (26%). One SP + A case consisted of 2 biopsies, one contained a solitary papilloma (left breast) and the other ADH (right breast). For purposes of this study, this case was classified as atypia outside of the papilloma.

### Outcome

Among the overall Mayo cohort, the relative risk of developing carcinoma was: NP 1.3 [95% confidence

**TABLE 1.** Comparison of Family History of Breast Cancer Across the Various Papilloma Subtypes

Diagnosis	Family History of Breast Cancer*			
	None N (%)	Weak N (%)	Strong N (%)	Total N (%)
No papilloma	2549 (52.60)	1119 (23.09)	911 (18.80)	4579 (94.49)
SP	105 (2.17)	50 (1.03)	41 (0.85)	196 (4.04)
SP + A	16 (0.33)	7 (0.14)	11 (0.23)	34 (0.70)
MP	18 (0.37)	6 (0.12)	6 (0.12)	30 (0.62)
MP + A	4 (0.08)	0 (0.00)	3 (0.06)	7 (0.14)

\*Family history was missing in 4262 cases.

**TABLE 2.** Frequency of Proliferative Breast Disease Across the Papilloma Subtypes

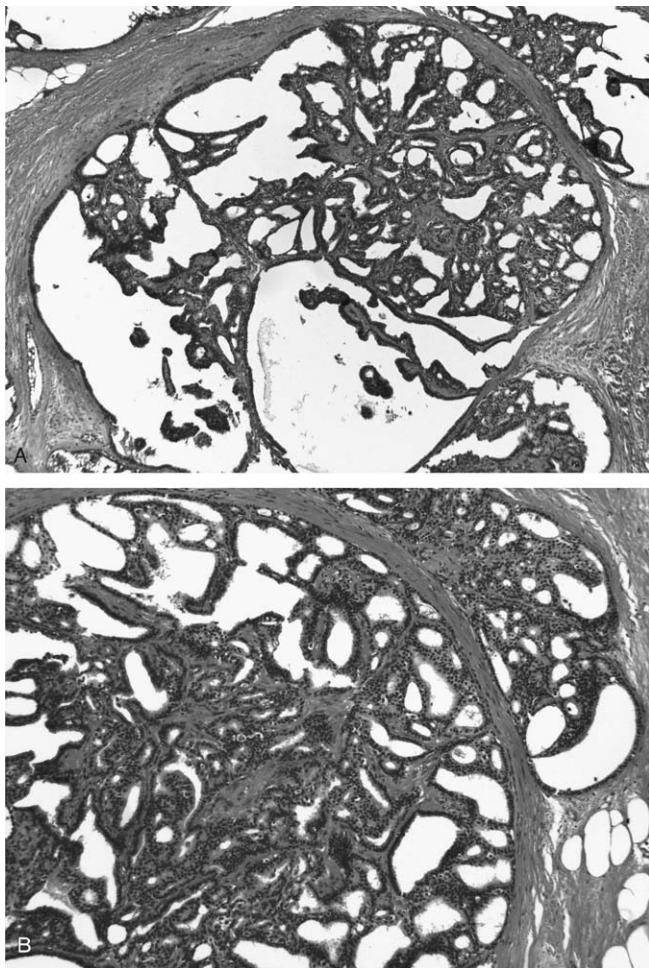
	Ductal Hyperplasia		Sclerosing Adenosis		Radial Scars	
	Yes N (%)	No N (%)	Yes N (%)	No N (%)	Yes N (%)	No N (%)
No papilloma*	1569 (18)	7055 (82)	2000 (23)	6625 (77)	339 (4)	8280 (96)
SP†	241 (64)	131 (36)	207 (56)	164 (44)	59 (16)	312 (84)
SP + A	52 (96)	2 (4)	38 (70)	16 (30)	18 (33)	36 (67)
MP	38 (93)	3 (7)	39 (95)	2 (5)	14 (34)	27 (66)
MP + A	13 (100)	0 (0)	8 (62)	5 (38)	4 (31)	9 (69)

Ductal hyperplasia includes moderate and florid ductal epithelial hyperplasia.

\*This includes NP, proliferative without atypia, and AH cases. Four observations missing values for ductal hyperplasia, 9 for radial scars, and 3 for sclerosing adenosis.

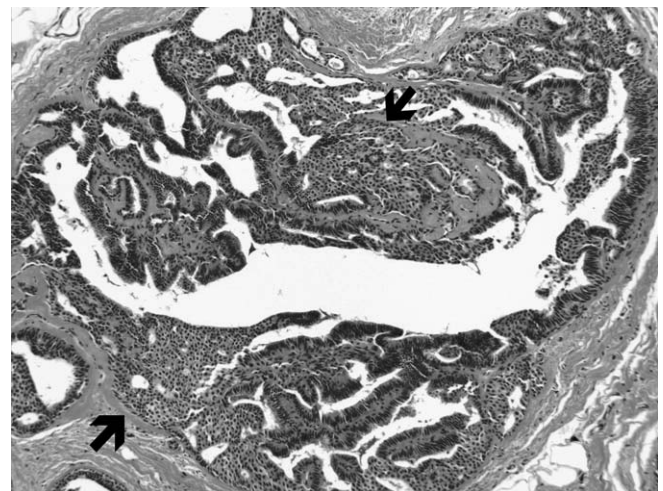
†One observation missing value for sclerosing adenosis and radial scar.

interval (CI), 1.2-1.4], PDWA 1.9 (95% CI 1.7-2.2), and AH 4.4 (95% CI 3.4-5.6). Overall, 724 (8%) of the patients in the cohort have developed breast carcinoma.

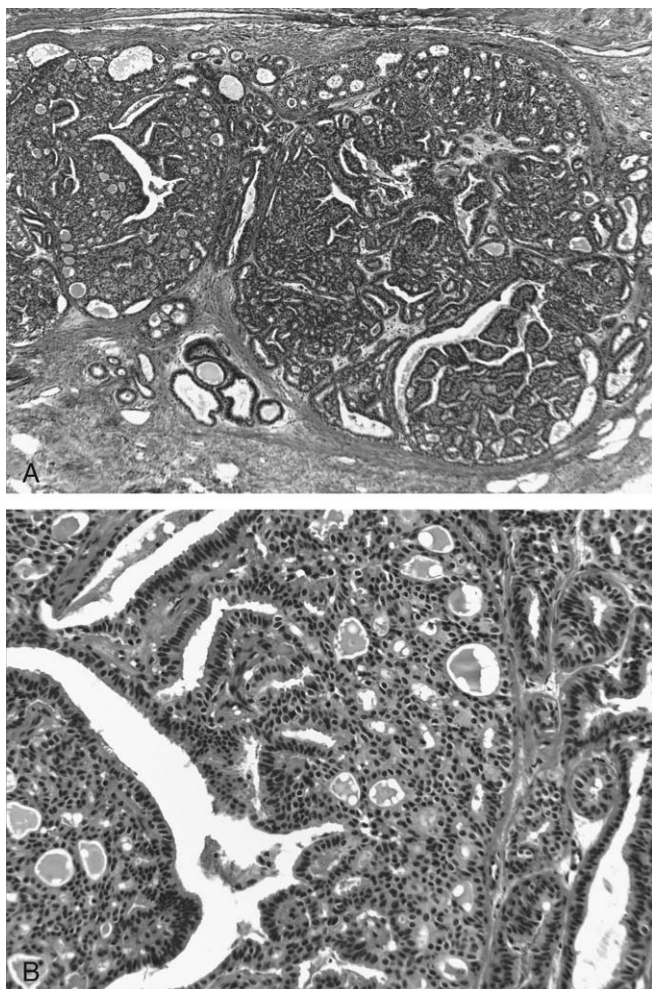


**FIGURE 1.** Low magnification scanning micrograph showing an architecturally complex papillary lesion containing fibrovascular stroma with focal cribriform growth. At higher magnification areas of cribriformlike architecture can be appreciated at the periphery of the lesion. Lack of uniform involvement and bland cytologic features preclude a diagnosis of ductal carcinoma in situ.

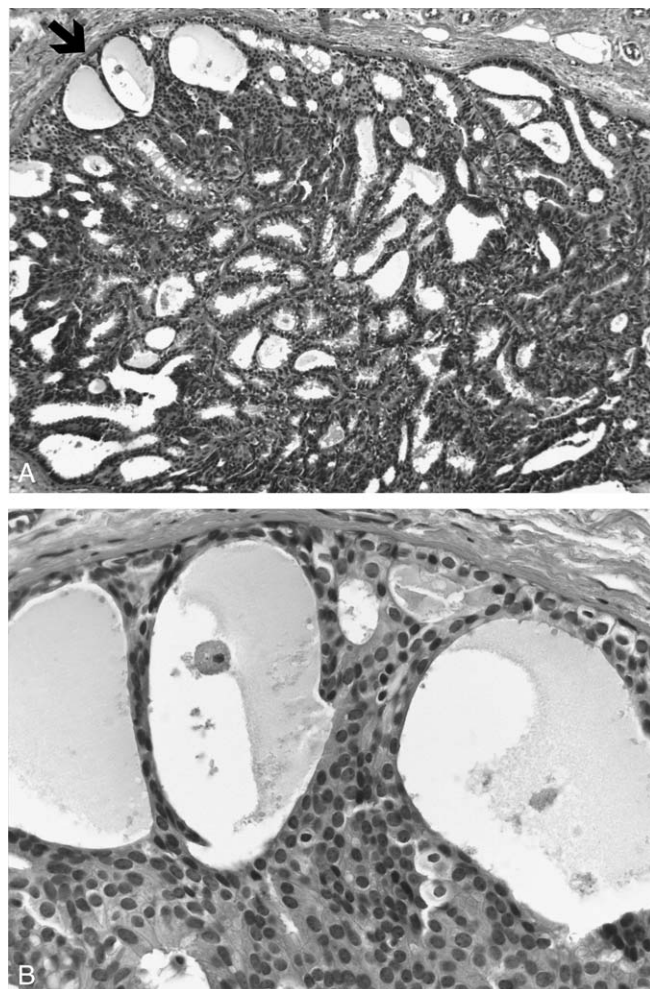
Table 3 summarizes the mean age at biopsy, mean interval to development of breast cancer, and risk of carcinoma development among the histopathologic groups with respect to papilloma status. Patients lacking a papilloma(s) who had proliferative disease without atypia had a relative risk of 1.90 (95% CI 1.66-2.16) of developing cancer compared to the control population. Patients with a solitary papilloma without atypia had a risk of 2.04 (95% CI 1.43-2.81), roughly equivalent to other forms of proliferative disease without atypia. Patients with AH and no papilloma(s) had a relative risk of 4.17 (95% CI 3.10-5.50). Individuals with a SP + A had a risk of 5.11 (95% CI 2.64-8.92), slightly greater than those with AH lacking a papillary lesion. The breast cancer risk for multiple papillomas without atypia fell between proliferative disease without atypia and AH (3.01, 95% CI 1.10-6.55). Multiple papilloma cases with atypia had the greatest likelihood of developing cancer, with a relative risk of 7.01 (95% CI 1.91-17.97). A Kaplan-Meier curve depicting cumulative incidence of breast cancer among all histopathologic groups is



**FIGURE 2.** Atypical papilloma (low magnification). Most areas are comprised of columnar epithelium on fibrovascular stalks. At least 2 foci (arrows) show a monotonous cellular proliferation lacking stroma and containing small secondary lumens.



**FIGURE 3.** At scanning magnification this papilloma is characterized by marked hypercellularity and variable architecture. Higher magnification photomicrograph highlighting confluent growth of epithelium with partially developed cribriform architecture.



**FIGURE 4.** The architecture is primarily microglandular, but focal complex growth may also be appreciated (arrow). Higher magnification of cribriform area. Lack of uniform involvement and low grade cytology preclude a diagnosis of in situ carcinoma.

presented in Figure 5. The mean interval to cancer development was greater than 5 years in all histologic groups except MP 4.8 (SD 3.2). It was longest in patients with NP 8.7 (SD 7.2). There was no difference in mean interval to cancer between AH and SP + A [6.5 (SD 5.3) vs. 6.2 (SD 4.7),  $P = 0.87$ ,  $t$  test].

As papillomas were most frequently identified in the setting of other proliferative lesions, we attempted to determine the degree to which the apparent breast cancer risk seen in these patients was directly attributable to the papilloma(s), versus other coexisting forms of proliferation present within the breast. We performed a univariate analysis using Poisson regression models subset to only those women with proliferative changes (ie, NP cases were excluded). After accounting for age and calendar period, women with some form of proliferative disease (eg, AH, radial scar, or sclerosing adenosis) and with a papilloma, had a relative risk of breast cancer roughly 20% higher

than those without a papilloma. Thus, within the group of patients with proliferative disease, the presence of a papilloma marginally increased risk. However, this result did not reach statistical significance ( $P = 0.17$ ). As the univariate model accounted for the excessive risk due to proliferative disease, but it did not adjust for the effects of other individual forms of proliferation, we performed a multivariate analysis adjusting for AH, ductal hyperplasia, sclerosing adenosis, and radial scars. Results were similar to the univariate model: the presence of papillomas increased risk by an additional 16% over those patients without a papilloma. However, this result again failed to reach statistical significance ( $P = 0.29$ ). We also performed the multivariate analysis subset to proliferative cases without atypia. After adjusting for sclerosing adenosis, radial scars, and duct hyperplasia, the presence of papilloma increased risk by an additional 10% over those patients without a papilloma ( $P = 0.42$ ).

**TABLE 3.** Demographic Characteristics and SIRs of Breast Carcinoma Development for the Mayo Cohort Compared With the Iowa SEER Registry

Diagnosis	Overall N	Age at Biopsy (y) Mean (SD)	Time to Cancer (y) Mean (SD)	Observed Cancers	Expected Cancers	SIR (95% CI)
No papilloma present						
NP	6053	49.9 (14.8)	8.7 (7.2)	383	298	1.28 (1.16-1.42)
Proliferative	2308	53.6 (12.1)	7.8 (6.4)	232	122	1.90 (1.66-2.16)
AH	267	57.3 (11.6)	6.5 (5.3)	50	12	4.17 (3.10-5.50)
Papilloma present						
SP	372	55.2 (14.5)	5.9 (5.0)	37	18	2.04 (1.43-2.81)
SP + A	54	59.1 (13.4)	6.2 (4.9)	12	2	5.11 (2.64-8.92)
MP	41	53.9 (15.5)	4.8 (3.2)	6	2	3.01 (1.10-6.55)
MP + A	13	65.1 (14.0)	5.8 (3.8)	4	1	7.01 (1.91-17.97)

The “Overall” column refers to the total number of cases in each group. Analyses account for the effects of age and calendar period.

In the SP + A patients, risk for breast cancer was not associated with the microscopic location of ADH. Cancers developed in 25% (4/16) of patients with ADH within papilloma compared with 29% (5/17) patients with ADH outside of the papilloma and 17% (2/12) with ADH in both locations. None of the patients with ALH alone (N = 6) has yet developed breast carcinoma.

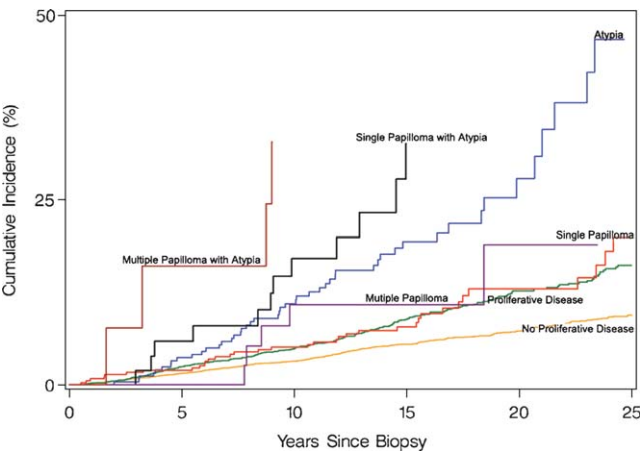
Table 4 summarizes side of cancer development in relation to the side of the original excisional biopsy. With respect to the overall Mayo cohort, 56% of the patients in the NP, PDWA, and AH groups developed carcinoma in the ipsilateral (same) breast as the biopsy.<sup>15</sup> Sixty-five percent of the carcinomas in the papilloma group developed in the ipsilateral breast. The likelihood for development of ipsilateral cancer among those with papilloma compared with nonpapilloma groups, was not statistically different ( $P = 0.33$ ,  $\chi^2$  test).

DISCUSSION

Our study defines the incidence, spectrum of pathology, and breast cancer risk attributable to benign

papillomas that were identified in a large cohort of consecutive, nonselected benign biopsies. It is the first to specifically address the cancer risk associated with papillomas, either with or without atypia, using epidemiologically valid comparisons between carefully defined pathologic subsets including nonpapilloma proliferative lesions. The data demonstrate that presence of a single papilloma without atypia conveys an overall breast cancer risk that is similar to or marginally greater than other commonly recognized proliferative fibrocystic lesions. It is unclear, even after multivariate statistical analysis, whether this small difference is due to the more frequent presence of other proliferative lesions in those with papillomas. Second, the presence of ADH/ALH in association with a single papilloma (“atypical papilloma”), does not appreciably modify the risk connotation attributable to atypia overall. Finally, the follow-up data from the cohort imply that patients with multiple papillomas are at a significantly elevated risk for breast cancer, even if atypia is not identified in their biopsy.

The Mayo benign breast disease cohort is derived from the surgical practice at one institution and is not



**FIGURE 5.** Kaplan-Meier curve illustrating cumulative incidence of cancer development among the histologic groups in the Mayo cohort.

**TABLE 4.** Breast Cancer Sidedness Among the Different Diagnostic Groups With Respect to Excisional Biopsy Location

Diagnosis	Number of Cancers*	Contralateral N (%)	Ipsilateral N (%)
All Subjects			
NP	354	163 (46)	191 (54)
Proliferative	234	107 (46)	127 (54)
AH	57	22 (30)	35 (61)
No papilloma present			
NP	354	163 (46)	191 (54)
Proliferative	194	93 (48)	101 (52)
AH	43	17 (40)	26 (60)
Papilloma present			
SP	34	10 (29)	24 (71)
SP + A	11	4 (36)	7 (64)
MP/MP + A	9	5 (56)	4 (44)

MP and MP + A cases were summed because of the small number of events. \*Individuals with missing side information, bilateral BBD, or bilateral cancer have been removed from the analysis.

enriched with extramural pathology consultation materials. Indications for breast biopsy were not different than those employed by comparable institutions during the survey period. To our knowledge, it is the largest benign breast disease cohort that has been subject to standard pathologic review.<sup>2,8–10,17,18,21,26</sup> The size of the cohort and long follow-up allow for robust statistical analysis. The observed proportion of cases and relative risks of carcinoma development associated with NP, proliferative, and atypical lesions is similar to other large surveys of benign breast disease.<sup>5,9,17,16,20,21</sup>

Benign papillomas constitute an important subset of mammary fibrocystic change, collectively accounting for about 5% of proliferative cases overall. As seen in this study, moreover, papillomas are frequently accompanied by a complex of other proliferative lesions, particularly adenosis and duct hyperplasia. Radial scars are also often present, especially in the SP + A, MP and MP + A subsets (31% to 34%). Finally, papillomas often comprise a background on which atypias develop; 20% of all atypias in our BBD cohort overall were present in cases that also had papillomas. Conversely, about 14% of biopsies with papilloma(s) contained ADH and/or ALH. The observed associations with adenosis and radial scarring are noteworthy in the sense that both are characterized by combined proliferation of epithelial and nonepithelial populations (myoepithelial cells and fibroblasts, respectively). These findings imply that breast tissues harboring papillomas may be characterized by simultaneous activation/proliferation of divergent cell populations. It may be hypothesized that the background of multilineage cell proliferation reflects an especially permissive environment for development of hyperplastic lesions, accounting for the markedly cellular character of this BBD subset, and possibly for the more frequent evolution of atypias.

Although papillomas are often associated with other proliferative or atypical lesions, it is nonetheless true that most benign biopsies with papillomas—about 78%—contained single papillomas and lacked ADH/ALH. Within this subset (ie, single papilloma without atypia) the age at diagnosis and overall breast cancer risk were quite similar to patients with proliferative lesions overall—53.6 versus 55.2 years and 1.90 (95% CI 1.66–2.16) versus 2.04 (95% CI 1.43–2.81), respectively. On the basis of these data we would not advise the risk classification of single, nonatypical papillomas apart from other proliferative fibrocystic lesions.

Some may hypothesize that atypical proliferations developing within papillomas represent biologically distinct, direct precursor lesions. However, the simultaneous presence of papilloma with ADH or ALH (SP + A) was associated with a breast cancer risk (5.11, 95% CI 2.64–8.92) that was similar to, or marginally elevated, relative to other atypias in our cohort (4.17, 95% CI 3.10–5.50). Importantly, in the papilloma cases we failed to identify significant tendency to ipsilateral breast cancer development or short interval to breast cancer diagnosis. Thus, we identify no convincing evidence to suggest that these lesions constitute an homogeneous group of direct cancer

precursors. We also attempted to discern whether the location of atypia relative to a papilloma had special significance. Although the number of cases limits definitive interpretation, our data imply that geographic location of ADH relative to a papillary lesion would not necessarily be a clinically useful indicator of breast cancer risk apart from other parameters. However, our data would not support the practice of separately denoting an atypical papilloma as a distinct subset of ADH.

Page et al<sup>25</sup> have published a nested case control survey that compares breast cancer risk in 122 patients with papillomas. Their incidence of atypia occurring within or in association with papilloma (17/122, 14%) was similar to our series (54/480, 11%) as was the time interval between biopsy and subsequent breast malignancy. However, the absolute risk of breast cancer after atypical papillomas in the Page et al study was 53% (9/17). In contrast, in our study only 22% of such individuals (12/54) had developed breast cancer at 16 years. They also observed a significantly elevated relative breast cancer risk (2.30 to 3.35 $\times$ ) attributable to papilloma that was modified by presence of concurrent atypia (4.40 to 13.10 $\times$ ). Because we employed similar diagnostic criteria, we ascribe the differences with our study to their relatively limited number of cases and the study design (ie, case control vs. cohort).

Haagensen<sup>12</sup> and Murad<sup>22</sup> have emphasized the unique clinical behavior of MP, noting from selected series of cases that these patients have significantly elevated breast cancer risk. The incidence and relative cancer risk of MP, however, has not been previously described. Our data show that MP cases constitute a rare subset, accounting for 0.6% of BBD patients. However, depending on the presence of atypical lesions, MP patients have a breast cancer risk that is 3 to 7 times greater than age matched women in the population overall. Thus, our data indicate that MP, even without concurrent atypia, convey a relative risk between proliferative disease overall and AH. On the basis of these findings, we recommend that MP should receive wider recognition as a diagnostic entity and that these patients should be, at a minimum, followed carefully.

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# Breast Cancer Prevention Strategies Explored

Tracy Hampton, PhD

**N**EW RESEARCH PRESENTED AT THE annual conference of the American Association for Cancer Research suggests that vitamin D and cyclooxygenase-2 (COX-2) inhibitors may help reduce the risk of breast cancer. The findings may help women, particularly those at elevated risk, take steps to prevent the development of this disease, although experts caution that any anticancer benefits of the COX-2 inhibitors will have to be balanced against the drugs' cardiovascular risks.

## THE SUNSHINE VITAMIN

Individuals who live in warm and sunny climates enjoy not only pleasant weather but also a decreased risk of developing some cancers. Epidemiological studies have demonstrated that there is higher mortality from colon, breast, and several other types of cancer in the northeastern United States than in the southwest and that the geographic distribution of these malignancies is related inversely to solar radiation (Grant WB. *Cancer*. 2002;94:1867-1875).

Because ultraviolet rays from sunlight trigger vitamin D synthesis in the skin, researchers presumed that this "sunshine vitamin" might play an important role in the reduction of cancer risk in sunnier regions. Now, two studies looking at breast cancer in particular add to the growing evidence that

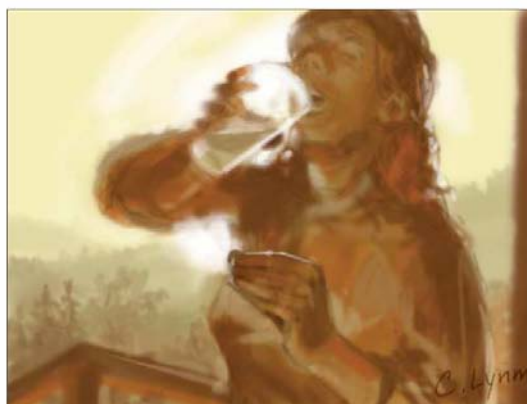
vitamin D can reduce a woman's risk of developing this disease.

In a study seeking to estimate the amount of vitamin D required to reduce the incidence of breast cancer, researchers performed a meta-analysis of two large previously published studies that measured vitamin D concentrations in the blood (as serum 25-hydroxyvitamin D) and subsequent breast cancer development (Lowe LC et al. *Eur J Cancer*. 2005; 41:1164-1169; Bertone-Johnson ER et al. *Cancer Epidemiol Biomarkers Prev*. 2005; 14:1991-1997).

"We found that women who consumed 1000 international units [IU] a day of vitamin D in addition to the normal background amount that women consume each day had a 10% lower risk of breast cancer," said lead author

Cedric Garland, DPH, of the University of California San Diego, in La Jolla.

In their analysis of 1760 individuals (including breast cancer patients and cancer-free controls matched on the basis of age, menopausal status, and other factors), Garland and colleagues plotted a dose-response gradient and found that as quintiles of serum 25-hydroxyvitamin D (0-11 ng/mL, 12-25 ng/mL, 26-31 ng/mL, 32-42 ng/mL, and >42 ng/mL) increased, the risk of breast cancer significantly decreased. A serum 25-hydroxyvitamin D concentration exceeding 52 ng/mL (which would require intake of more than 2700 IU/d of vitamin D<sub>3</sub> in an individual weighing 70 kg) was associated with 50% lower risk of breast cancer compared with a serum concentration of less than 12 ng/mL.



New studies suggest that vitamin D and cyclooxygenase-2 (COX-2) inhibitors may help reduce a woman's risk of developing breast cancer, although the potential benefits of the latter will need to be weighed against their cardiovascular risks.





The authors noted that the US median intake of 320 IU/d of vitamin D is only about one tenth of the amount found to be associated with a 50% reduction of breast cancer incidence. They concluded that increasing daily intake of vitamin D, perhaps by fortification of foods, should be considered. "We believe that higher doses of vitamin D will produce proportionate reduction in the incidence of breast cancer," said Garland.

Researchers in Canada also are providing additional evidence of the potential of vitamin D to decrease breast cancer risk. In a population-based, case-control study in Ontario, women with breast cancer (identified through the Ontario Cancer Registry) and controls are being interviewed about their past and present diet and sun exposure. Investigators have conducted a preliminary analysis of 576 women aged 20 to 59 years diagnosed from July 1, 2003, to June 30, 2004, and 813 controls of the same age range.

The researchers found evidence for a sun exposure-related reduction in breast cancer risk associated with ever working in an outdoor job, as well as engaging in a number of different outdoor activities between the ages of 10 and 29 years. Greater reductions were associated with participating in larger numbers of activities at a younger age. Also associated with reduced risk was taking cod liver oil for 10 years or more and consuming more than 9 glasses of milk per week compared with fewer than 5 glasses at ages 20 to 29 years.

The investigators' preliminary analysis suggests that earlier exposures to vitamin D may be more important for reducing breast cancer risk than recent exposures, noted lead author Julia Knight, PhD, of Mount Sinai Hospital, in Toronto, Ontario. "A major finding was that many of these factors are most important during the ages 10 to 19," said Knight. "It seems that exposure to vitamin D around the time that the breasts are developing during adolescence may be particularly important," she added.

Studies exploring how vitamin D may reduce breast cancer have revealed that

it regulates cell proliferation and differentiation, cell death, and other processes important for maintaining homeostasis (Welsh J. *Am J Clin Nutr*. 2004;80(6 suppl):1721S-1724S).

#### COX-2 INHIBITORS

While COX-2 inhibitors such as rofecoxib and celecoxib have been under fire for increasing the risk for adverse cardiac events, researchers in the oncology community have been pointing to the potential of these drugs as anticancer agents. Because COX-2 is an enzyme that is produced in higher amounts in a variety of cancers, it may be a potential molecular target for chemoprevention strategies.

A recent study led by Lynn Hartmann, MD, of the Mayo Clinic College of Medicine, in Rochester, Minn, supports this, finding that high levels of COX-2 expression is associated with a significantly greater likelihood of subsequent breast cancer in women with atypical hyperplasia, a condition in which abnormal and increased numbers of cells are present. In previous research, Hartmann and colleagues found that women with atypical hyperplasia have a 4-fold increased risk of developing breast cancer (Hartmann LC et al. *N Engl J Med*. 2005;353:229-237). "So they're clearly a high-risk group," said Hartmann. "Can we apply a molecular strategy . . . to try to separate out who in fact will go on to develop breast cancer?" she asked.

Hartmann and collaborators reasoned that COX-2 seemed like an especially good candidate as a biomarker for cancer. "That enzyme is a rate-limiting step in a number of important processes required for carcinogenesis: reduced apoptosis, reduced functioning of the immune system, increased angiogenesis," she explained.

To test COX-2 as a biomarker, the investigators used data and samples from the Mayo Clinic Benign Breast Disease Cohort of 9343 women who had an open breast biopsy between 1967 and 1991. Formalin-fixed, paraffin-embedded tissue from 247 women with atypical hyperplasia was

assessed for COX-2 expression by immunohistochemistry; expression was scored on a scale from 0 (negative) to 3+ (high intensity). Scores for the samples were 0 for 28 samples (11.3%), 1+ for 113 samples (45.8%), 2+ for 74 samples (30%), and 3+ for 32 samples (13%).

Forty women developed breast cancer over a median follow-up period of 15 years, and significantly higher COX-2 staining intensity was found in the atypical hyperplasia cells of these individuals compared with those of women who did not go on to develop the disease. "For those that were negative or only negligibly staining, at 20 years 14% of those women had developed breast cancer. If there was strong staining by COX-2, 31% of those women had gone on to develop breast cancer," said Hartmann.

Because COX-2 may be important for the development of some breast cancers, it might also make a good target for treatment strategies. To see if COX-2 inhibitors have anticancer properties, Randall Harris, MD, PhD, of the Ohio State University School of Public Health, in Columbus, and colleagues conducted a case-control study comparing the effects of selective and nonselective COX-2 inhibitors on breast cancer incidence. Questionnaires on past and current use of prescription and over-the-counter medications and breast cancer risk factors were collected from 323 women with breast cancer and 629 cancer-free controls.

Results showed significantly lower rates of breast cancer associated with use of selective COX-2 inhibitors as a group, regular aspirin, and ibuprofen or naproxen, with the lowest rates among women taking selective COX-2 inhibitors. Acetaminophen, which has negligible COX-2 activity, and low-dose aspirin (81 mg) were not associated with a change in risk.

If future studies support the association of COX-2 inhibitors and reduced breast cancer risk, then the drugs' potential benefits will need to be weighed against the cardiovascular risks. "The rule of thumb is first do no harm," said Harris. □

## **COX-2 expression in atypia: correlation with breast cancer risk.**

Lynn C. Hartmann, Wilma L. Lingle, Marlene H. Frost, Shaun D. Maloney, Robert A. Vierkant, V. Shane Pankratz, Thea Tlsty, Amy C. Degnim, Daniel W. Visscher  
*Presented at 97<sup>th</sup> American Association for Cancer Research Annual Meeting, April 1-5, 2006, Washington, D.C.*

### Background

Women with atypical hyperplasia have a significantly increased risk of a later breast cancer (RR~4.0) and are excellent candidates for chemoprevention strategies. Identification of appropriate molecular targets is a priority. COX-2 is up-regulated in a variety of malignancies by several oncogenic mechanisms. Increased COX-2 expression has been documented in DCIS specimens.<sup>1</sup> We sought to determine COX-2 expression in women with atypia and assess possible correlations with a later breast cancer.

### Methods

The Mayo Clinic Benign Breast Disease Cohort includes 9343 women who had an open breast biopsy between 1967 and 1991.<sup>2</sup> For 247 women with atypical hyperplasia, there was formalin-fixed, paraffin-embedded tissue available for assessment of COX-2 expression by immunohistochemistry. Our study pathologist (DWV) scored the COX-2 expression on a scale from 0 (negative) to 3+ (high intensity). We used Cochran-Mantel-Haenszel tests for trend to compare intensity of staining in the samples of women who developed breast cancer (cases) to that of women who did not develop breast cancer (controls).

### Results

Forty of the 247 women with atypia have developed breast cancer over a median follow-up of 15 years. The atypia samples displayed a range of COX-2 expression with values of 0 for 28 (11.3%), 1+ for 113 (45.8%), 2+ for 74 (30%), and 3+ staining for 32 (13%). We found significantly higher COX-2 staining intensity in the atypias of those women who went on to develop breast cancer compared to the controls who did not (p=0.04).

### Conclusions

Women with atypia are recognized as having a high risk for a later breast cancer. Intense COX-2 expression is associated with a significantly greater likelihood of a subsequent breast cancer in women with atypia and represents one potential molecular target for chemoprevention strategies.

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## **Assessment of the Gail model in a cohort of women with atypical hyperplasia**

V. Shane Pankratz, Robert A. Vierkant, Shaun D. Maloney, Marlene H. Frost, Daniel W. Visscher, Lynn C. Hartmann. Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905. (AACR 2006.)

### **Background**

Understanding an individual woman's risk of developing breast cancer is of high importance if we are to tailor clinical management properly. We sought to evaluate the performance of the Gail model<sup>1</sup> in a cohort of women with atypical hyperplasia, and to determine if other histopathological features might contribute to enhanced risk prediction in this cohort.

### **Methods**

The Mayo Clinic Benign Breast Disease (BBD) Cohort includes 9343 women who had an open breast biopsy between 1967 and 1991.<sup>2</sup> Of these, 336 women had atypical hyperplasia, a group with significantly increased risk of a later breast cancer (RR~4.0). Gail model and other risk factors were obtained via survey and medical record review. Lifetime risk (thirty-year probability) of breast cancer was computed for each woman. Logistic regression was used to assess the concordance between the predicted and observed lifetime risk. Proportional hazards regression, with bootstrap model selection, was used to identify a potential risk prediction model for this high-risk group of women.

### **Results**

In this atypia sub-cohort, 64 women experienced a breast cancer with an average follow-up of about 15 years. This number of events was slightly lower than the number predicted by the 30-year Gail model probabilities (rate ratio [95% CI] = 0.94 [0.74 – 1.20]). At the individual level, the concordance between observed and predicted breast cancer was 0.59, and did not reach statistical significance (0.13). The model selection process identified one covariate that was associated with breast cancer risk in this sub-cohort: the number of foci of atypia.

### **Conclusions**

On average in this atypia cohort, the Gail model prediction was accurate, but the per-individual concordance between observed and predicted breast cancer was low. Knowledge of the number of foci of atypia provided additional information about breast cancer risk. The development of alternative risk models in this group, and in the entire BBD cohort, are in process.

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### **The Impact of Lobular Involution on Breast Cancer Risk.**

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*Background:* Lobular involution is a histologic finding that reflects atrophy associated with physiologic aging in the human breast. Based on epidemiologic associations, involution has been hypothesized to have relevance in breast tumorigenesis.

*Methods:* A breast pathologist examined benign breast biopsies of 8,743 women in the Mayo Benign Breast Disease cohort and classified them according to the degree of lobular involution as follows: none (0%), partial (1-74%), or complete (>75%). Each benign biopsy was also evaluated per standard criteria as nonproliferative (NP), proliferative disease without atypia (PDWA), and atypical hyperplasia (AH). Age at biopsy, family history of breast cancer, and development of breast cancer were obtained from medical records or questionnaires (17-year mean follow-up). Associations of involution with other breast cancer risk factors were carried out using chi-square tests and logistic regression analyses. Relative risks of breast cancer were estimated by comparing the number of observed events with the number expected based on rates from the Iowa SEER registry.

*Results:* Distribution of the patients by the three levels of involution was as follows: none-1,628 (18.6%); partial-5,202 (59.5%); and complete-1,913 (21.9%). Increased involution was found to correlate with increased age and decreased family history of breast cancer. The relative risk of breast cancer was significantly lower in patients who had complete (0.91, 95% CI 0.74-1.10) compared to those with partial (1.45, 95% CI 1.32-1.59) or no involution (1.88, 95% CI 1.59-2.21) ( $P<0.001$ ). Age and family history modified breast cancer risk. In patients with PDWA, the relative risk for women with no involution was (2.94, 95% CI 2.26-3.75), while that for women with complete involution was only (1.11, 95% CI 0.68-1.72) ( $P<0.001$ ). The relative risks in patients with NP and AH displayed similar associations.

*Conclusions:* The degree of lobular involution correlates inversely with breast cancer risk. It modifies breast cancer risk in patients stratified by age, family history, and type of histology. These data indicate that aberrant or delayed involution is a biologically important constitutional variable in breast cancer biology.

## Analysis of Cancer Risk in Women with Radial Scars of the Breast

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**Background:** Radial scars (RS) are characterized by an elastotic central core containing entrapped tubules that radiate outward in a stellate manner. The epithelial component may show varying degrees of proliferation. Previous studies have shown that RS impart an increased risk of breast cancer development.

**Design:** Radial scars were systematically identified in a histopathologically defined benign breast disease (BBD) cohort of 9073 patients biopsied between 1967 and 1991. Overall histology was classified as nonproliferative (NP), proliferative disease without atypia (PDWA), or atypical hyperplasia (AH) per standard criteria. The presence, number, and size of RS were counted for each case. The relative risk of cancer development within the BBD cohort was compared to that expected in the general population using standardized incidence ratios (SIRs, mean follow-up interval 17 years).

**Results:** RS were identified in 441 (4.9%) of the cohort cases; 384 (87%) of these contained one RS, 42 (9.5%) contained two, nine (2%) contained three, and six (1.5%) contained four or more, with a maximum of 11. RS size information was available in 434 cases. The majority of RS (357/434, 82%) were less than 5mm in diameter; 61 biopsies (14%) contained from 5-9.9mm RS; and 16 (4%) had RS 10mm or greater in diameter. Tables 1 and 2 compare the relative risk of BBD subsets defined by presence, size, and number of RS to patient groups lacking RS.

### Incident Breast Cancer Relative Risk

Diagnosis	Number Eligible Women	Relative Risk (95% CI)
NP	6048	1.07 (0.96, 1.18)
PDWA	2311	1.57 (1.37, 1.80)
PDWA + RS	377	1.84 (1.33, 2.49)
AH	273	4.01 (3.03, 5.21)
AH + RS	64	3.33 (1.67, 5.97)

### Relative Risk By Radial Scar Feature

Feature	Number Eligible Women	Relative Risk (95% CI)
1 Scar	384	2.02 (1.48, 2.69)
2+ Scars	57	2.12 (0.85, 4.35)
Size < 5 mm	357	1.84 (1.32, 2.51)
Size > 5 mm	77	2.50 (1.20, 4.61)

**Conclusions:** RS imparts no increased breast cancer risk compared to other forms of PDWA (i.e. duct hyperplasia and/or adenosis). Likewise, RS associated with AH also connotes no increased risk above that of AH. Breast cancer risk was not modified significantly by the size or number of RS lesions.

## Epidemiologic Comparison of Disease Incidence Among Populations: The Person-Years Approach

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In epidemiological studies it is often of interest to compare disease incidence within a study cohort to that of a reference population. The person-years approach is often used to make and summarize such comparisons. The resulting Standardized Mortality Ratios (SMRs) summarize the degree to which observed cohorts differ from the reference population. While there have been criticisms of this method, there are few alternatives when one wishes to compare study groups with respect to their degree of deviation from population-based expectations. Our study of this topic is motivated by a desire to study the risks of breast cancer in women with a history of a benign breast biopsy relative to a reference population. In this study, follow-up data were obtained from a consecutive series of 9086 women having had a benign breast biopsy. Women with a history of a benign breast biopsy had an SMR of 1.6 (95% CI: 1.5 – 1.7). We present an overview of the person-years method and demonstrate how estimates of per-subject expected events may be used in the place of group-aggregated expected events. We also outline modifications that may alleviate concerns that arise in the use of this approach, motivated by data from the study that motivated our investigations.

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